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9. The methods of statistical analysis should be described in detail sufficient that a knowledgeable reader could reproduce the analysis if the data were available.

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* *From* Methodologic Guidelines for Reports of Clinical Trials (Cancer Treat Rep 69:1–3, 1985).

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567 ANNOUNCEMENTS

570 CORRECTION

Cisplatin, Doxorubicin, and Cyclophosphamide Chemotherapy for Advanced Endometrial Carcinoma[1,2]

M. M. Turbow,[3] S. C. Ballon,[3,*] B. I. Sikic,[3] and M. M. Koretz[4]

Nine of 19 patients (47%) with widespread or recurrent endometrial carcinoma responded to chemotherapy with cisplatin, doxorubicin, and cyclophosphamide. Two complete clinical responses and seven partial responses were achieved. A "second-look" laparotomy documented the complete response in one patient. The addition of cisplatin to doxorubicin and cyclophosphamide increased toxicity without increasing the antitumor activity previously reported for the two-drug combination. Performance status had a marked influence on response, while sites of metastases, amount of residual disease, and histologic grade did not affect the response rate. A schema for the treatment of patients with endometrial carcinoma with progestins and/or cytotoxic chemotherapy is suggested. [Cancer Treat Rep 69:465–467, 1985]

Endometrial carcinoma is the most common invasive cancer of the female genital tract, with 38,000 newly diagnosed cases in 1980 (1). Despite its prevalence, however, experience with cytotoxic chemotherapy is limited because early endometrial cancer often is cured with surgery and irradiation, while advanced or recurrent disease frequently responds to progestational therapy (2). There are relatively few controlled investigations of cytotoxic drugs in the management of these patients; however, doxorubicin and cyclophosphamide both have produced temporary remissions in some patients with metastatic or recurrent endometrial cancer (3–6). A recent study evaluated doxorubicin and cyclophosphamide in 11 patients with metastases refractory to progestins. Objective responses were seen in five patients, including one complete response lasting 1 year (7). Because of the reported activity of cisplatin on other malignancies of the female reproductive organs, the Northern California Oncology Group chose to evaluate the combination of cisplatin, doxorubicin, and cyclophosphamide in patients with histologically confirmed advanced or recurrent endometrial carcinoma.

MATERIALS AND METHODS

From September 1978 to July 1981, 21 patients with biopsy-proven advanced or recurrent endometrial carcinoma received cisplatin (60 mg/m^2), doxorubicin (50 mg/m^2), and cyclophosphamide (600 mg/m^2) administered iv every 4 weeks. No patient had received prior cytotoxic chemotherapy; however, eight had received progestational agents. Patients with a history of ischemic heart disease, congestive heart failure, or creatinine clearance < 50 ml/minute were excluded. Cisplatin was administered as a 24-hour infusion in approximately 75% of patients, while the remainder received a 2-hour infusion in an attempt to shorten their stay in the hospital.

A partial response (PR) was defined as at least a 50% reduction in the product of the perpendicular diameters of a measurable lesion for at least 1 month, without growth of other measurable lesions or the appearance of new lesions. A clinical complete response (CCR) was defined as the total disappearance of all lesions clinically and/or radiographically. Patients in CCR after 1 year of combination chemotherapy were considered for "second-look" laparotomy. Treatment was discontinued in those patients in whom CR was confirmed at laparotomy. Progressive disease was defined as growth of any lesion and resulted in cessation of this combination.

Dosages of all three drugs were initially reduced by 25% for patients who had had prior radiation therapy.

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[2]Supported in part by Public Health Service grant CA-25862 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

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Doses were subsequently reduced for myelosuppression or impaired renal function. Doxorubicin was administered to a total dose of 450 mg/m^2, at which point patients were continued on cisplatin and cyclophosphamide.

RESULTS

Patient characteristics are shown in table 1. The mean age of the 21 patients was 65 years. Almost 50% of the patients had a Karnofsky performance status (PS) of 90%-100%; however, 75% had residual disease measuring > 2 cm in diameter. Four of the 21 patients had papillary carcinoma of the endometrium, a recently described subtype associated with a poor prognosis (8). Most tumors were poorly differentiated. Whereas only eight of the patients had received progestational therapy, 17 had undergone prior irradiation.

Nineteen of the 21 patients were evaluable for response to chemotherapy. Two patients had no measurable tumor at the time of entry in this study. They remained clinically and radiographically free of tumor for 6 and 10 months following initiation of chemotherapy but were not considered evaluable for response. The overall response rate to the combination was 47%, with two patients (11%) achieving a CCR and seven (37%) achieving a PR. In one patient, CR was confirmed at second-look laparotomy. The median durations of response were 3 and 8 months, respectively, for the partial and complete responders. The median survival was 10 months for all evaluable patients.

Both PS and prior therapy appeared to influence response (table 2). Responses were seen only in patients with a Karnofsky PS of 80%-100%; both patients who experienced CCR were at the 100% level. All four of the patients who had not received either progestins or radiation therapy responded, as did eight of the 12 patients (75%) who had received no prior progestational therapy. Only one patient previously treated with progestins responded. The relationship between PS and prior therapy was examined. Eleven of the 14 patients (79%) with a PS of 80%-100% had received no prior progestational therapy. In contrast, only one patient in the impaired PS group (50%-70%) had not received progestins. The association between prior progestational therapy and poor PS indicates that patients treated initially with progestins began cytotoxic chemotherapy later in the course of their disease.

The number of involved sites did not influence the rate of response; however, two patients with isolated pulmonary metastases responded to chemotherapy. Neither histologic grade nor amount of residual disease affected the response rate. One of the four patients with papillary serous carcinoma achieved a PR. The rate of administration of cisplatin had no effect on response.

Only one patient underwent second-look laparotomy and had no residual tumor found at surgery. She had received irradiation but no prior progestational therapy and began chemotherapy with suboptimal (> 2 cm) residual disease located in the pelvis and abdomen.

Overall, toxicity was as expected. Seven patients had total wbc counts between 1000 and 2000/mm^3; however, only two had counts < 1000/mm^3. Only three patients experienced moderate thrombocytopenia (50,000-90,000 cells/mm^3). Significant myelosuppression occurred only in patients who had previously received irradiation. Vomiting was severe in 50% of patients. One patient developed doxorubicin cardiomyopathy. Neuropathy was not encountered. Only mild, transient azotemia was observed.

TABLE 1.—Patient characteristics

Characteristic	No. of patients
Eligible	21
Mean age in yrs (range): 65 (45-72)	
Karnofsky PS	
50%-60%	3
70%-80%	9
90%-100%	9
Residual disease (cm)	
< 2	5
⩾ 2	16
Pathologic type	
Adenocarcinoma	17
Papillary carcinoma	4
Histologic grade	
Well-differentiated	3
Moderately differentiated	3
Poorly differentiated	12
Unknown	3
Prior radiotherapy	
Yes	17
No	4
Prior progestational therapy	
Yes	8
No	13

TABLE 2.—Response associations

	No. of responders*
Karnofsky PS	
80%-100%	9/14 (64)
50%-70%	0/5 (0)
Prior hormone therapy	
No	8/12 (67)
Yes	1/7 (14)
Prior radiotherapy	
No	4/4 (100)
Yes	5/15 (33)

*No. of responders/No. of patients fully evaluable for response in each category (%).

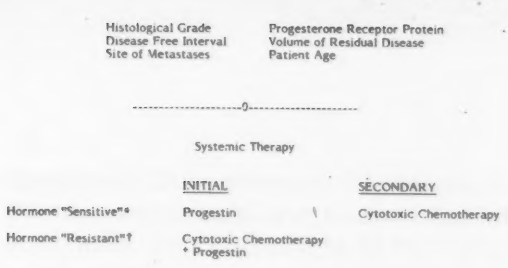

FIGURE 1.—Factors influencing response to progestational therapy. * Well-differentiated tumor, long interval from initial diagnosis to recurrence, recurrence in nonirradiated site (lung), PRP+, low volume of disease, young patient. † Anaplastic tumor, short interval from initial diagnosis to recurrence, recurrence in previously irradiated tissue (pelvis), PRP-, high volume of disease, elderly patient.

DISCUSSION

Several drugs have been reported to produce response rates of 20%-30% when given as single agents to patients with endometrial carcinoma (3). Higher response rates have been noted with combination chemotherapy in small groups of patients. Cohen et al (9) treated seven patients with a combination of melphalan, 5-FU, and megestrol acetate and noted responses in six. All seven patients treated by Bruckner and Deppe (10) responded to a combination of cyclophosphamide, doxorubicin, 5-FU, and megestrol acetate. Muggia et al (7) described five of 11 patients responding to doxorubicin and cyclophosphamide; one of these responded for > 12 months. Thus, the 47% response rate in this uncontrolled series of patients treated with cisplatin, doxorubicin, and cyclophosphamide is not superior to that achieved by doxorubicin and cyclophosphamide without the addition of cisplatin.

Several observations can be made regarding the use of cytotoxic chemotherapy in patients with widespread or recurrent endometrial carcinoma. As in patients with other tumor types, PS is an important prognostic factor influencing response to systemic chemotherapy. In this study, responses were observed only in those patients with a Karnofsky PS of 80%-100%. As noted in other studies, histologic grade and site of metastases did not affect the response to chemotherapy. Although prior progestational therapy appeared to influence the response rate in this study, most of these patients also had a markedly impaired PS.

In view of the observed response rate and the possibility of long-term control in some patients with the use of cytotoxic chemotherapy, a re-evaluation of our present treatment approach toward patients with metastatic or recurrent endometrial carcinoma is warranted. Treatment with a progestin has been considered first-line therapy for this group of patients, with cytotoxic drugs being initiated subsequently for those patients who fail to respond to progestins. Factors which influence the responsiveness of endometrial cancer to progestins include histologic grade, patient age, volume of residual disease, site of metastases, and disease-free interval (11). Unfortunately, information regarding the levels of progesterone receptor protein (PRP), another potentially useful predictor of response, is not available in most patients with endometrial carcinoma (12). Patients who have a profile which correlates with a decreased likelihood of response to progestins should be considered for initial treatment with cytotoxic agents alone or in combination with a progestin. If patients with little likelihood of response are given a 3-month trial of progestins, subsequently administered cytotoxic chemotherapy could possibly be less effective as the disease progresses and PS becomes impaired.

Based on these observations, one can formulate a schema for the treatment of patients with recurrent or widespread endometrial carcinoma (fig 1). If the tumor contains PRP and/or there are other factors indicating the tumor is likely to respond to hormonal manipulation, progestins should be initiated. If the tumor is negative for PRP and/or other factors indicate that the tumor probably will not respond to hormonal therapy, cytotoxic chemotherapy should be considered either prior to or together with the institution of progestins.

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Combined Chemotherapy (Vindesine, Lomustine, Cisplatin, and Cyclophosphamide) and Radical Radiotherapy in Inoperable Nonmetastatic Squamous Cell Carcinoma of the Lung[1,2]

T. Le Chevalier,[3,*] R. Arriagada,[3] P. Baldeyrou,[3] M. Martin,[4] P. Duroux,[5] A. Jacquotte,[3] H. Sancho-Garnier,[3] and J. Rouesse[3,6]

Thirty-three evaluable patients with locally advanced squamous cell carcinoma of the lung were entered in a phase II study combining chemotherapy (vindesine, lomustine, cisplatin, and cyclophosphamide) and radical radiotherapy. Fourteen patients had an objective response rate of 42% (two complete responses and 12 partial responses) with the first two cycles of chemotherapy. All patients received radiation therapy to the primary tumor, mediastinum, and supraclavicular nodes, and responders to chemotherapy received four additional cycles. On final evaluation, 18 patients (54.5%) achieved complete response and six patients (18%) achieved partial response. The objective response rate was 73% with the combined therapy. The median survival was 15.9 months. Toxicity was acceptable. We conclude that our results justify a phase III study comparing combined treatment versus radiotherapy alone. [Cancer Treat Rep 69:469–472, 1985]

Squamous cell carcinoma represents about 35% of lung cancer and its curative treatment is surgical. However, in most cases patients are inoperable at the time of presentation, either because of metastatic disease or because of locally advanced tumor which is not resectable.

Fifty percent to 75% of patients with locally advanced squamous cell carcinoma of the lung (LASCCL) will die from intrathoracic complications related to the primary tumor, often without distant metastases (1,2). The median survival of such untreated patients is 3–5 months (3). With radical radiotherapy there was a complete radiologic response in 45%–65% of patients with LASCCL (4,5). However, long-term survival remains disappointing because of distant metastases (6). Increased survival requires both local control and prevention of metastatic dissemination. With this goal in mind, we developed a phase II study combining radical radiotherapy and systemic chemotherapy containing vindesine, lomustine, cisplatin, and cyclophosphamide (VCPC).

PATIENTS AND METHODS

Patients entered in this study were previously untreated and had histologically proven squamous cell carcinoma of the lung. They were deemed inoperable by bronchoscopic, x-ray, or physical examination. Eligibility requirements are as follows: measurable tumor; Karnofsky performance status > 50% and age ⩽ 70 years; no prior chemotherapy or radiation therapy; no previous history of malignant disease (except basal cell skin carcinoma); no cardiac, liver, or renal abnormalities contraindicating the combined modality therapy; no esophageal or chest wall involvement and no superior vena cava syndrome requiring urgent radiation therapy; chest x-ray and fiberoptic bronchoscopy; normal bone scan; normal brain scintigraphy or computerized tomographic (CT) scan; normal liver CT, scintigraphy, or ultrasound; and disease limited to one hemithorax, mediastinum, and supraclavicular nodes, provided that all nodes could be included in the same radiotherapy fields as the primary tumor. The treatment schedule (fig 1) had two monthly cycles of the VCPC regimen with radiographic, fiberoptic, and histologic examinations performed in all patients 3 weeks after the second cycle to determine the initial response to chemotherapy. All patients then received split-course irradiation to a port including primary

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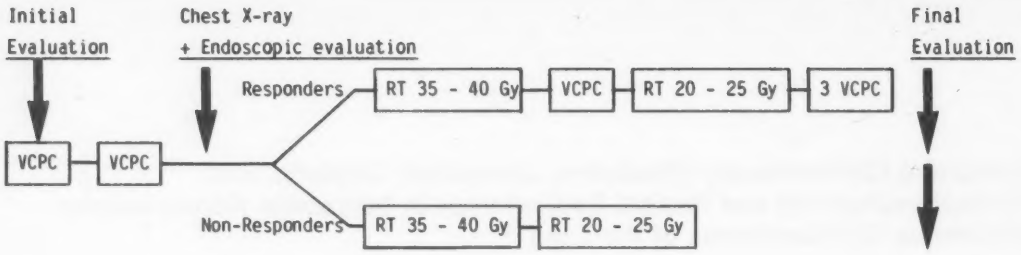

FIGURE 1.—Treatment schedule: vindesine (1.5 mg/m^2 on Day 1); lomustine (50 mg/m^2 on Day 2); lomustine (25 mg/m^2 on Day 3); cisplatin (100 mg/m^2 on Day 3); and cyclophosphamide (200 mg/m^2 on Days 2-4).

tumor, mediastinum, and bilateral supraclavicular nodes. A total radiation dose of 6000 rad, with appropriate spinal shielding, was planned to be delivered to all patients. The sequential scheme of the technique included is as follows:

(*a*) Two large opposed anteroposterior/posteroanterior beams, complex shaped, used to irradiate the whole target volume including tumor volume, homolateral hilar, and mediastinal and supraclavicular lymph nodes. The dose delivered with this first-beam arrangement was limited to 4000 rad in 16 fractions.

(*b*) Two opposed oblique beams including the tumor and homolateral mediastinal lymph nodes. The dose delivered with this second-beam arrangement was 2000 rad in eight fractions.

It was assumed that patients responding to chemotherapy could benefit from a regular administration of chemotherapy and that split-course irradiation was as effective as continuous irradiation (7). Patients who demonstrated a response to the first two cycles of chemotherapy resumed VCPC for one cycle between the first and second series of radiotherapy and for three more cycles after radiation therapy. A final assessment identical to the initial assessment was performed 4 months after the end of radiotherapy, to determine combined modality response.

Thirty-three patients (all male) were entered in the study from January 1980 to June 1982, and all were evaluable for response. The median age was 56.3 years (range, 32-70) and the median Karnofsky performance status (PS) was 80% (range, 60%-100%). Squamous cell carcinoma was well-differentiated in 17 patients, moderately differentiated in five patients, and poorly differentiated in 11 patients. Two patients were considered unresectable because of major respiratory deficiency (one T_2N_1 and one T_2N_x). The remaining 31 patients were unresectable because of local extension (16 T_3, 26 N_2); six had supraclavicular node involvement. Radiation therapy was delivered with cobalt 60 γ-ray beams in 11 patients and with 18-MV photon beams in 22 patients; the mean equivalent radiation dose, estimated by the TDF method (8), was 5900 rad.

Response criteria were as follows: *complete remission (CR)*—the complete disappearance of all objective tumor on chest x-ray and optically and histologically at the time of fiberoptic bronchoscopy without evidence of new disease; *partial remission (PR)*—a decrease of $\geq$ 50% of the product of the two largest perpendicular diameters of radiologically measurable disease with concurrent endoscopic verification and no evidence of new disease; *minor response*—a decrease of 20%-50% of the product of the two largest perpendicular diameters of radiologically measurable disease with concurrent endoscopic verification and no evidence of new disease; *stable disease*—no radiologic and endoscopic change and no evidence of new disease; and *progressive disease*—radiologic or endoscopic progression or evidence of new disease.

RESULTS

Among the 33 patients, the radiologic and endoscopic examination after two cycles showed two with CR and 12 with PR (42% objective response), ten with minor response (30%), seven with stable disease (21%), and two with progressive disease (6%). Twenty-two of 33 patients resumed chemotherapy (two responding patients did not receive further chemotherapy: one because of hematotoxicity, the other refused). On final evaluation, 18 patients (54.5%) achieved CR and six (18%) achieved PR; the objective response rate was 73% for the combined modality treatment. Three patients (9%) obtained a minor response and three had stable disease. Two patients had disease progression and one died during therapy. At final evaluation, the mean PS was 77%; 12 patients had increased their PS and 11 were unchanged.

The overall survival rate was 97% at 6 months, 70% at 12 months, and 35% at 18 months. The median survival was 15.9 months. Median survival of responders to chemotherapy was 17.4 months versus 14.6 for nonresponders. Median survival of the 18 complete responders was 17.8 months.

The overall incidence of distant metastases was 33% (11 of 33 patients). Among the 18 patients achieving CR, five had local recurrence, three had distant metastases,

and three had local recurrence plus distant metastases (mean time to recurrence, 15.4 months). Twenty-four of 33 patients died, 20 of progressive disease, one of possible toxicity, and three of intercurrent disease. Nine patients are still alive: six of these are disease-free 14, 19, 20, 24, 24, and 35 months after initiation of the therapy.

The toxicity of this regimen was acceptable. Fourteen patients had no toxicity to chemotherapy and only two patients had World Health Organization grade 4 hematologic toxicity (one with leukopenia, one with anemia and thrombocytopenia). All patients had nausea and vomiting with cisplatin courses, symptomatically treated with metoclopramide. Nine patients had dysphagia after the first radiation course and two had infectious bronchopneumonia (one after the first radiation course and one after the second radiation course).

Late toxicity was manifested as pulmonary fibrosis in five patients (symptomatic in two) and increase in respiratory deficiency in one patient. One patient died of myocardial infarction with no history of antecedent heart disease.

DISCUSSION

Locally advanced tumors treated with systemic therapy will predictably fail to respond. Moreover, even after adequate radiation therapy, unresectable lung carcinoma will often recur in distant sites. Local control and treatment for occult metastasis are required if the objective is curative therapy. Combination chemotherapy has resulted in objective response rates in inoperable squamous cell carcinoma of the lung (9–13) and has shown survival benefit in responders in some studies (9,11,13). However, combination chemotherapy does not appear to adequately control the primary tumor. On the other hand, radiation therapy offers a chance of local control with doses > 5500 rad (5).

Many studies have demonstrated a benefit to combined modality therapy in different sites of locally advanced tumors (14) but the risk of added toxicity often limits the benefit obtained with each modality and potentially results in diminution of the desired synergistic effect. This may, at least in part, result from the need to reduce effective doses of each modality to avoid unacceptable toxicity.

In LASCCL, primary chemotherapy is given with the objective to treat occult metastases but also, possibly, to decrease the tumor bulk to obtain better local control with radiotherapy. Most of the studies of combined modality therapy in LASCCL report chemotherapy given after radiation therapy. Theoretically, these protocols lose the benefit of chemoreduction of tumor bulk at the primary site (15–17). Samuels et al (18) reported a benefit for responders to chemotherapy (median survival, 70 vs 26 weeks for nonresponders), with a schedule similar to that of the present study.

In our trial, a 42% response rate is obtained with the combination chemotherapy. This result is equivalent to that obtained with other vindesine and cisplatin combinations (13,19). The moderate doses employed in this regimen permit delivery of adequate radiation doses (6000 rad) which led to a 54.5% CR rate that was histologically confirmed.

The acceptable toxicity of this combined modality therapy, the CR rate, and the median survival of 15.9 months justify a randomized trial comparing combined treatment to radiotherapy alone. We have recently undertaken such a phase III study to better define the role of combined modality therapy in LASCCL.

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Treatment of Metastatic Malignant Melanoma With Intensive Melphalan and Autologous Bone Marrow Transplantation[1,2]

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Twenty patients with metastatic malignant melanoma were treated with melphalan (180–225 mg/m² iv over 3 days) followed by re-infusion of previously harvested and cryopreserved autologous bone marrow. Granulocyte (> 500/μl) and platelet (> 20,000/μl) count recovery occurred a median of 14 days (range, 12–22) and 19.0 days (range, 12–28) after bone marrow transplantation, respectively. Three patients died due to hemorrhage or infection. One of these patients and another (who ultimately achieved a complete response), both with brain metastases, developed intralesional hemorrhage when the platelet count was only moderately reduced. Complete responses occurred in six patients and ranged from 4 to 14 months, with a median of 5 months. Six patients achieved a partial remission lasting 2–5 months. High-dose melphalan and autologous bone marrow transplantation are a promising therapy for patients with metastatic malignant melanoma. [Cancer Treat Rep 69:473–477, 1985]

The therapy for patients with disseminated malignant melanoma is generally unsatisfactory. Dimethyl-triazeno-imidazole-carboxamide (dacarbazine), the most active chemotherapeutic agent used for treating these patients, yields an objective response rate of 8%–31%, with a duration of response of 4 months (1–7). A disproportionately large number of these responses occurred in female patients with disease limited to the skin or lymph nodes, a more favorable group of patients (1,4,6). Attempts to combine dacarbazine with other agents and combination chemotherapy regimens have not conclusively demonstrated improved response or survival rates compared to dacarbazine alone (4–6, 8–13).

Recent studies have demonstrated that augmented doses of iv melphalan administered with autologous bone marrow transplantation have been associated with significant antitumor responses (14,15). Here we report our treatment of 20 patients with metastatic malignant melanoma using high-dose iv melphalan and autologous marrow transplantation.

MATERIALS AND METHODS

Between January 1, 1980 and December 31, 1983, 20 patients with metastatic malignant melanoma were treated at Case Western Reserve University, Cleveland, OH; Washington University, St. Louis, MO; Vanderbilt University, Nashville, TN; and Baylor University Medical Center, Dallas, TX. This protocol was approved by the review board for human investigation at each institution, and all patients gave informed consent. Patients had histologic evidence of malignant melanoma and had received neither melphalan nor pelvic radiotherapy. Patient evaluation included: medical history, physical examination, chest x-ray, tumor measurement studies, complete peripheral blood count, and serum tests of liver

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(SGOT, LDH, alkaline phosphatase, total and direct bilirubin, albumin, and total protein) and renal function (BUN and creatinine). The latter three tests were obtained two to three times weekly during hospitalization, and monthly after transplantation.

At the time of bone marrow harvest and cryopreservation, patients had normal bone marrow biopsies and had not had chemotherapy for a period of at least 4 weeks before the marrow harvest. The marrow was frozen using previously described techniques, and was stored in the liquid phase of a liquid nitrogen refrigerator until used (16).

Melphalan (100-mg vials) was provided by the National Cancer Institute and was reconstituted according to the package instructions, using 1.0 ml of the acid-alcohol solvent and 9.0 ml of diluent provided by the manufacturer (Burroughs-Wellcome, Research Triangle Park, NC).

Antiemetic medications were administered before each dose of melphalan and as needed thereafter. The drug was given as a bolus injection into a fast-running iv infusion daily for 3 consecutive days. The dose of melphalan referred to in this paper represents the total of 3 consecutive days of drug administration. For example, a dose level of 180 mg/m^2 means that the patient received 60 mg/m^2 iv daily for 3 consecutive days. Patients received only a single course of intensive therapy and marrow reinfusion; second courses or any form of maintenance therapy was not given. Two patients treated at the 225-mg/m^2 dose level received cyclophosphamide (300 mg/m^2) iv 7 days before the first dose of melphalan to theoretically reduce gastrointestinal toxicity (17). All blood products were irradiated with 3000 rads to prevent graft-versus-host disease. Frozen bone marrow was transported to the bedside in a liquid nitrogen refrigerator for reinfusion 3 days after the final dose of melphalan. The marrow was thawed in a 37°-40°C water bath and rapidly injected iv without washing or filtering steps. Side effects noted during marrow infusion were transient, and commonly included mild nausea and occasional emesis, dry cough, and flushing. The odor of the dimethylsulfoxide metabolite excreted by the lungs persisted for 1-2 days.

After the transplant, tumor measurement studies were repeated at intervals of every other month. Complete response (CR) was defined as the disappearance of all signs and symptoms referable to tumor. Partial response (PR) was defined as a $> 50\%$ reduction in cross-sectional tumor area without the appearance of new lesions; a $< 50\%$ decrease in tumor size was defined as no response. Time intervals were calculated from Day 0, the day of marrow transplantation. Follow-up is current as of March 1, 1984.

RESULTS

Patient characteristics at the time of transplantation are shown in table 1. Ages ranged from 30 to 63 years (median, 43.5) for the 20 patients. Fifteen patients were treated at the 180-mg/m^2 dose level, and five received 225 mg/m^2, two of whom received cyclophosphamide priming (300 mg/m^2 iv 1 week before melphalan). The dose of bone marrow re-infused ranged from 1.0 to 2.2 × 10^8 nucleated bone marrow cells/kg (median, 1.4 × 10^8/kg).

Toxic effects associated with high-dose iv melphalan have been described in detail previously (15). Briefly, patients who received melphalan at a dose of 225 mg/m^2 experienced more stomatitis, esophagitis, and diarrhea than those who received melphalan at 180 mg/m^2. Myelosuppression, although attenuated by autologous marrow transplantation, was severe. Peripheral blood neutrophil count was $> 500/\mu l$ a median of 14 days (range, 12-22) after marrow infusion. The platelet count (without the support of transfusion) was $> 20{,}000/\mu l$ within 12-28 days (median, 19.0) after transplantation. Patients 2 and 8 with brain metastases developed intracranial hemorrhage into these metastatic lesions (confirmed on computerized tomographic brain scan) when the platelet counts were $96{,}000/\mu l$ and $55{,}000/\mu l$, respectively. Patient 2 had hemorraghic malignant ascites at this time as well. Because of increased intracranial hemorrhage during therapy, Patient 2 died, while Patient 8 achieved CR and regained normal neurologic function. Patient 5 had documented brain metastases with intralesional hemorrhage before initiating any therapy when the platelet count was $381{,}000/\mu l$. She experienced additional bleeding problems when her platelet count was $41{,}000/\mu l$ after the intensive autografting regimen. She died 1 month after transplantation from progressive disease.

The rate of recovery of peripheral blood counts was the same, regardless of the dose of melphalan or whether patients received pretreatment with cyclophosphamide. One patient developed palmar erythema and rash, believed to be due to melphalan. There were no pulmonary, cardiac, or renal sequelae attributable to melphalan in high dose.

Six of 20 patients achieved CR, with a median duration of 5 months (range, 4-14). Additionally, six patients achieved PR lasting 2-5 months (median duration, 3). Of the remaining eight patients, four died within 5 weeks of transplantation due to hemorrhage or infection and four failed to respond to therapy. All five patients receiving melphalan at 225 mg/m^2 responded (four CR and one PR), compared to seven of 11 who received 180 mg/m^2 (two CR and five PR). Overall, 12 of 20 patients undergoing high-dose melphalan therapy responded.

Fifteen patients had previously received chemotherapy, and objective responses to high-dose melphalan and autografting were noted in nine (four CR and five PR). Five patients received only local radiation therapy or surgery before they were treated with transplantation, and two CR and one PR were noted. There was no statistical

TABLE 1.—Patient characteristics

Patient No.	Sex	Age (yrs)	Previous therapy*	Sites of metastases						Dose of melphalan (mg/m^2)	Antitumor response/ duration (mos)†	Survival
				Skin	Node	Liver	Lung	Brain	Other			
1	M	39	1 → 4			+		+		180	ED	3 wks
2	M	54	2 → 4	+	+	+	+	+	+‡	180	ED	1 wk
3	F	59	2 → 3	+			+			180	PR/3	7 mos
4	M	31	2		+	+	+		+‡	180	ED	3 wks
5	F	31	4				+	+		180	NR	1 mo
6	F	31	1			+				180	PR/5	6 mos
7	M	30	1 → 3 → 2	+	+	+	+	+	+‡	180	NR	3 mos
8	F	60	3	+			+	+		180	CR/5	6 mos
9	M	45	5		+					180	NR	3 mos
10	M	32	5 → 6 → 1				+		+‡	180	CR/9+	9+ mos
11	F	43	5		+					225¶	CR/14	24+ mos
12	M	33	6 → 1	+	+		+		+‡	180	NR	2 mos
13	F	50	3	+	+	+	+			180	PR/2	3 mos
14	F	58	5		+					225	CR/6	10 mos
15	F	37	2		+					225	CR/4	8 mos
16	M	43	1 → 5 → 4	+		+				180	PR/2	3 mos
17	M	37	1		+	+	+			180	PR/3	4+ mos
18	F	47	4 → 1	+	+		+			225	CR/8	14 mos
19	F	63	5	+	+	+			+§	225¶	PR/3	4 mos
20	M	44	5 → 2		+	+			+‡	180	ED	3 wks

* 1 = single-agent chemotherapy; 2 = combination chemotherapy (conventional); 3 = previous autologous bone marrow transplantation; 4 = radiation therapy; 5 = surgery; 6 = immunotherapy.

†ED = early death; NR = no response.

‡Omentum or other intra-abdominal disease.

§Mediastinum.

¶Includes cyclophosphamide priming.

difference in response rate between patients who were treated with chemotherapy before transplant and those who were not. Nine patients with visceral organ involvement responded to therapy (six PR and three CR). Four patients (Nos. 9, 11, 14, and 15) had lymph node metastases as the sole site of involvement by melanoma. Patient 9 did not respond to therapy, while the other three patients achieved CR. Patient 11 achieved a 14-month CR. She relapsed in the involved groin nodes but received local radiation therapy, and has had no evidence of disease 10 months later.

DISCUSSION

Myelosuppression is presumably the dose-limiting toxicity for most antineoplastic agents such as melphalan, yet greater antitumor effects may be observed when more drug is administered (18-21). Bone marrow transplantation enables the administration of greater doses of drug than can be achieved without marrow re-infusion.

In early studies, conventional doses of melphalan were reported to give a 17% response rate in malignant melanoma (four PR and CR in 24 evaluable patients) (22). Ariel and Pack (23) treated 31 patients with malignant melanoma using melphalan (100 mg) iv followed by noncryopreserved autologous bone marrow re-infusion. They noted objective responses in 11 patients. In later studies, McElwain et al (14,24), Millar et al (17), and Hedley et al (25) used doses of melphalan up to 215 mg/m^2 iv to treat patients with malignant melanoma. Most patients received two courses of therapy, and CR were noted in only one of eight patients. Corringham and co-workers (26) treated three patients with metastatic melanoma with autologous marrow re-infusion and 120-200 mg/m^2 of iv melphalan. Two patients did not respond and one received adjuvant therapy after resection of involved lymph nodes. Compared to these studies, our higher response rate may be explained by the fact that we infused melphalan in divided doses over 3 days. Administering the melphalan over 3 days would have more antitumor effect on theoretical grounds, since more cells would be in cycle over a longer time period (27).

This report describes intensive therapy, and three patients died as a result of hemorrhage or infection before recovery of marrow function. The antitumor responses, however, were striking in some cases. Tumor necrosis and subsequent bleeding into the tumor resulted in substantial morbidity in two patients. These patients did not have evidence of infection or disseminated intravascular coagulation. This phenomenon may relate to plasminogen activator elaborated by the tumor (28). Melanoma cells cultured in vitro have been shown to elaborate fibrinolytic activity which could potentiate intralesional bleeding in vivo during periods of thrombocytopenia. Melanoma patients receiving high-dose melphalan therapy may need aminocaproic acid or platelet transfusions at higher platelet counts than other thrombocytopenic cancer patients (29). Malignant melanoma metastatic to visceral organs, including the liver and brain, can respond to intensive melphalan and autologous marrow transplantation. Despite cerebrospinal fluid drug concentrations of only 0.15-0.20 μg/ml, parenchymal brain metastases can respond to therapy (15).

The response rate we observed (six PR and six CR in 20 patients) is significantly higher than that reported with conventional doses of chemotherapeutic drugs alone or in combination. Although the responses tended to be of brief duration, this may reflect the fact that only a single course of therapy was administered. Combining melphalan with other agents for synergism or repeating the intensive autografting procedure may have longer lasting antitumor effect.

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Cisplatin and Etoposide Combination Chemotherapy for Refractory Small Cell Carcinoma of the Lung[1,2]

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Twenty-nine patients with refractory recurrent small cell carcinoma of the lung were treated with cisplatin (40 mg/m²) and etoposide (200 mg/m²) each day for 3 days, repeated every 3–4 weeks. Fifteen of these patients had received etoposide in their original treatment regimen. Fifteen (52%) of all patients had a major response, as did nine (60%) of the patients with prior exposure to etoposide. Myelotoxicity was moderately severe. The median duration of responses was 3 months (range, 6–36 weeks). This study suggests synergism between cisplatin and etoposide. The toxicity seen in this heavily pretreated group of patients suggests that smaller doses be studied in this group. The synergism may be best utilized in the initial regimens against small cell lung cancer. [Cancer Treat Rep 69:479–481, 1985]

Small cell lung carcinoma (SCLC) is an extremely resistant neoplasm after it has become refractory to primary chemotherapeutic regimens. Responses to "salvage" chemotherapy with either single agents or drug combinations have been few and of brief duration (1). Although etoposide is considered to be one of the most active agents against SCLC, it has a poor record when used as a single agent in refractory disease (2–4).[4] Cisplatin is also ineffective in most patients when used as a single agent in refractory SCLC (5–9).

Preliminary reports of small numbers of patients indicate that the combination of etoposide and cisplatin is active against resistant SCLC. Lopez et al (10) reported five responses (one complete and four partial) in eight previously treated patients using etoposide at 150 mg/m² on Days 1, 2, and 3 and cisplatin at 20 mg/m² on Days 1–5 every 3 weeks. Tinsley et al (11) obtained complete or partial responses in seven of 15 patients (47%) with resistant SCLC using a similar dose schedule. Osoba et al (12) used etoposide and cisplatin in 22 patients with resistant disease, and obtained two complete and eight partial responses.

Here we report 29 patients with refractory SCLC treated with etoposide and cisplatin, using slightly higher doses than were previously studied. The objectives of this study were twofold: (*a*) to confirm previous reports of the activity of this combination in larger numbers of patients; and (*b*) to evaluate the activity of this combination in patients who had previously received etoposide as a component of initial therapy.

PATIENTS AND METHODS

All patients with recurrent or refractory SCLC were eligible for this study, including those who had been previously treated with etoposide. Other criteria for entry included a serum creatinine < 2.0 mg/dl, a wbc count > 4000/mm³, a platelet count > 125,000/mm³, an estimated survival of 4 weeks, and a performance status of 0–2 (Zubrod).

Cisplatin was administered at 40 mg/m² iv in normal saline infused over 1 hour on Days 1, 2, and 3. Vigorous saline hydration was accomplished to keep urine output at least 150 ml/hour for the 3-day period. In an attempt to take advantage of its schedule dependency, etoposide (200 mg/m²) was given on Days 1, 2, and 3 as hourly infusions. Cycles were to be repeated every 3 weeks and continued until disease progression was demonstrated or toxicity was intolerable. Complete response was defined as complete clinical disappearance of known areas of neoplastic disease. Partial response required a > 50% reduction in all measurable lesions for at least 4 weeks. Mini-

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mal response was defined as an objective decrease of < 50% in the size of measurable lesions.

RESULTS

Twenty-nine patients (21 male; eight female) were treated with etoposide and cisplatin as described. Three patients died early and were inevaluable for response. Two of these patients died suddenly at home, and the cause could not be ascribed definitely to either progressive cancer or drug toxicity; one died of sepsis while leukopenic following the first course. Fifteen of 29 patients (52%) had major responses (complete or partial); 11 (36%) had minimal response or no response to therapy. The median number of months of chemotherapy administered in the original regimens was 5 (range, 2–14). The median number of drugs in these regimens was four (range, three to six), and the median number of months off chemotherapy before relapse was 5 (range, 0–22).

Of 15 patients who had previously been treated with etoposide as a component of initial therapy, nine (60%) had good responses (one complete and eight partial). The original regimens containing etoposide are described in table 1. The cumulative dose of previously administered etoposide was not related to the response to salvage therapy. Three of the nine responders had progressed while receiving an etoposide-containing regimen immediately prior to treatment with etoposide/cisplatin. The remaining six patients had received etoposide at an earlier time during primary therapy. The median time from the initial therapy to relapse or progression was 10 months (range, 2–36).

For the entire group of 29 patients, the responses to etoposide/cisplatin salvage therapy were usually brief and ranged from 6 to 36 weeks (median, 12). Median survival from the time of starting salvage therapy for the entire group was 4 months.

The major toxicity produced by this salvage regimen was myelotoxicity, which was severe. Of 88 courses of therapy, only 52 (59%) could be given at 100% of the planned dose. All patients received a full first dose, but only 23 of 59 repeat courses (38%) could be administered at full dose. Wbc count nadirs < 1000/mm^3 or platelet count nadirs < 15,000/mm^3 were seen in ten patients (35%). Nine of the 15 patients who had either complete or partial responses had marked myelosuppression at some time during their therapy. Fifteen of 59 repeat courses of treatment (25%) were delayed because of myelosuppression. Hypomagnesemia developed in most patients who received more than two courses of therapy. Magnesium supplementation did not usually correct the hypomagnesemia, but the patients were usually not symptomatic. Nausea and vomiting was usually mild to moderate, and all patients received high-dose metoclopramide and dexamethasone during cisplatin administration. All courses were administered in the hospital, yet seven patients (24%) required additional hospitalization for treatment-related toxic effects.

DISCUSSION

Synergism between etoposide and cisplatin has been demonstrated in the mouse P388 leukemia model (13). Similar therapeutic synergism has also been suggested in patients with resistant germinal neoplasms. The combination of etoposide and cisplatin has produced durable complete responses in 20%–25% of patients resistant to vinblastine, bleomycin, and cisplatin (14). Treatment with single-agent etoposide, cisplatin, or other combinations produced only rare complete responses in the past for this group of patients with resistant tumors.

The achievement of a 50% major response rate in patients with refractory SCLC is superior to results reported with previously tested salvage regimens, and confirms the activity of the etoposide/cisplatin combination. Our response rate is consistent with those reported in other smaller series (9–11). Evans et al (4) reported 34 evaluable patients with refractory small cell carcinoma treated with 20 mg/m^2 of cisplatin each day for 5 days and 100 mg/m^2 of etoposide for 3 days (except for five patients who received 40 mg/m^2 of cisplatin on Day 1 only). A 44% major response rate with this lower dose of cisplatin and etoposide appears to produce a similar response rate to that which we report, and with less myelotoxicity. The smaller dose may be better therapy for these refractory patients, yet the best dose for response in previously untreated patients is not known at this time. In addition, the achievement of a 60% response rate in patients previously treated with etoposide provides further evidence for the synergy of etoposide and cisplatin against SCLC.

Substantial myelotoxicity was not unexpected in this group of heavily pretreated patients. The possible syner-

TABLE 1.—Patients previously treated with etoposide

Original regimen	No. of— Patients	No. of— Responses to etoposide/cisplatin
CAV/etoposide*	3	3
High-dose cyclophosphamide/etoposide†	5	3
CA/etoposide‡	4	3
CAV-HP/etoposide§	3	0

* 300 mg/m^2 of etoposide iv every 3 wks with CAV (cyclophosphamide, doxorubicin, and vincristine).

†400 mg/m^2 of etoposide iv × 3 with high-dose cyclophosphamide for 2 cycles.

‡50 mg/m^2 of etoposide iv × 5 with each 3-wk cycle of CA (cyclophosphamide, doxorubicin).

§200 mg/m^2 of etoposide iv on Days 1 and 8 of cycle × 3 as consolidation after 6 cycles of CAV. HP = hexamethylmelamine; procarbazine.

gistic effect of these drugs may also have affected the bone marrow. Because of this toxicity, and the relatively short duration of response, we feel that a lower dose of etoposide should be used to decrease myelosuppression in this group. The observed activity of etoposide/cisplatin against refractory SCLC suggests that the optimal use of this combination may be in initial chemotherapy combinations. Limited data are available concerning primary treatment with etoposide and cisplatin-containing combinations. Sierocki et al (15) and Natale and Wittes (16) reported 38 patients who were treated with cisplatin (60 mg/m^2) on Days 1 and 22, followed by 3 days of etoposide (120 mg/m^2) on Days 4, 6, and 8 and on Days 25, 27, and 29. These patients then received four courses of cyclophosphamide, doxorubicin, and vincristine every 3 weeks, and then the entire program was recycled. The responses obtained in these patients were rapid and invariably maximum by the end of the induction with cisplatin and etoposide. Patients with limited-stage disease had a 100% response rate (52% complete responders) versus an 88% response rate (41% complete responders) in patients with extensive-stage disease. Median survivals were 22 months in limited-stage patients, and 11 months in those with extensive disease. Stoller et al (17) described eight of nine responses (89%) using etoposide (200 mg/m^2) on Days 1 and 2, and cisplatin (100 mg/m^2) on Day 1 in previously untreated patients. Kim and McDonald (18) also described a 91% response rate in 11 limited-stage patients and an 84% response rate in 13 extensive-stage patients treated with cisplatin (75 mg/m^2) on Day 2, and etoposide (125 mg/m^2) on Days 1, 3, and 5 every 4 weeks.

These results suggest that the combination of etoposide and cisplatin, in combination with other active drugs or possibly in alternating non-cross-resistant schedules, may improve initial therapy in patients with SCLC. More intensive treatment may be possible without prohibitive toxicity in previously untreated patients.

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Etoposide and Cisplatin With or Without Bleomycin as First-Line Chemotherapy for Patients With Small-Volume Metastases of Testicular Nonseminoma[1]

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Between December 1981 and September 1982, a phase II study of etoposide and cisplatin was carried out in 17 patients with small-volume metastases of testicular nonseminoma to see whether the toxicity associated with bleomycin could be avoided without loss of therapeutic effect. Of 16 evaluable patients followed for 13–23 months (median, 18.5), four failed to achieve complete remission and three had disease progression. Conversely, all 18 equivalent patients treated with bleomycin, etoposide, and cisplatin between January 1981 and November 1982 (observation time, 12–34 months; median, 25) achieved complete remission and have been continuously disease-free since treatment ($P = 0.07$). The results suggest that bleomycin contributes significantly to the combination chemotherapy for testicular nonseminoma, and although its omission led to a marked reduction in toxicity, loss of therapeutic activity was also apparent. Prolongation of the intervals between cycles of etoposide and cisplatin from 3 to 4 weeks may significantly reduce the effectiveness of the two-drug combination, whereas no evidence of this was seen in patients treated with bleomycin, etoposide, and cisplatin. [Cancer Treat Rep 69:483–488, 1985]

Following reports of the activity of etoposide as a single agent or in combination with cisplatin with or without bleomycin for the salvage treatment of testicular nonseminoma in patients relapsing after first-line chemotherapy (1-7), we have investigated a combination of bleomycin, etoposide, and cisplatin (BEP) and demonstrated it to be an effective first-line treatment (8,9). With BEP, as with other regimens such as PVB (cisplatin, vinblastine, and bleomycin) (10,11), tumor volume is a major determinant of the outcome of treatment with chemotherapy; patients with small-volume metastases have a particularly favorable outcome (8). In this group of patients an important objective is to reduce the toxicity of chemotherapy without losing therapeutic effectiveness. Although our experience suggests that the hematological toxicity of PVB can be reduced by substituting etoposide for vinblastine, the pulmonary, cutaneous, and vascular complications of bleomycin remain. To reduce toxicity further, bleomycin was omitted, and a combination of etoposide and cisplatin (EP) was investigated in a pilot study, with the aim of proceeding subsequently to a prospective randomized trial comparing EP with BEP in good-risk patients. The present report describes our experience in 17 patients with limited-volume disease treated with EP between December 1981 and September 1982 and compares the findings with the outcome of treatment in 18 patients with comparable-stage disease treated with BEP between January 1981 and November 1982.

PATIENTS AND METHODS

All patients had histologically proven nonseminomatous germ cell testicular tumors.

Staging included lymphangiography, computerized tomographic (CT) scanning of the chest and abdomen, ultrasonic scanning of the retroperitoneum and liver, iv urography, measurement of serum alpha-fetoprotein (AFP) and beta-human chorionic gonadotropin (beta-HCG) levels, liver function tests, and measurement of renal clearance. The Royal Marsden staging classification (8) was employed.

Chemotherapy

Etoposide and Cisplatin

Seventeen patients received etoposide at a dose of 120

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mg/m^2 iv on Days 1-3 and cisplatin at a dose of 20 mg/m^2 on Days 1-5 of each cycle, with a planned interval of 3 weeks between the first day of each cycle. Renal clearance (EDTA) was measured before each course of treatment and cisplatin was given with iv hydration as described elsewhere (9).

Bleomycin, Etoposide, and Cisplatin

Eighteen patients received BEP. The schedule was as above, with the addition of bleomycin at a dose of 30 mg iv on Days 2, 9, and 16 of each cycle.

When the BEP protocol was initiated in 1979, etoposide was given on Days 1-5, but this was modified to Days 1-3 because of myelotoxicity (9). In the present BEP series, four of 18 patients received 5 days of etoposide: two patients for two cycles, one patient for four cycles, and one patient for one cycle.

Monitoring, Reassessment, and Postchemotherapy Management

Serum AFP and beta-HCG levels were monitored throughout treatment. Patients were reassessed after four cycles of chemotherapy unless persistent elevation of serum markers gave an earlier indication of failure to respond, in which case chemotherapy was changed (this occurred in two patients receiving EP). Reassessment after four cycles of chemotherapy included CT scanning of the chest and abdomen. Until September 1981, treatment protocols included postchemotherapy irradiation for selected patients. No patient receiving EP was irradiated but four of 18 receiving BEP received radiotherapy. Patients with residual masses present after chemotherapy with or without radiotherapy were considered for surgery. Masses were resected after EP in two of 17 patients and after BEP in two of 18 patients.

Criteria for Patient Entry and Selection of Chemotherapy

Criteria for Entry to the Studies

All patients had histologically proven nonseminomatous germ cell testicular malignancy. Patients were in the following stage categories: IM, IIA, IIB, IIIA, IIIB, IVO/AL$_1$, and IVBL$_1$. Thus, patients either had no clinical evidence of metastases other than raised serum markers or retroperitoneal node metastases $\leq$ 5 cm in diameter with not more than three lung metastases. The only exception was one patient receiving EP who was treated without clinical evidence of disease and with normal markers because tumor had extended through the tunica albuginea into the scrotal sac. In this patient the risk of relapse was felt to be high, and a close surveillance policy (12) was impractical for social reasons.

Selection of EP or BEP

Patients receiving BEP (January 1981-November 1982) either presented with small-volume stage II, III, or IV disease (14 patients) or relapsed after surveillance for stage I disease (four patients) (12,13). Of the 18 patients receiving BEP, preliminary details on ten have been reported previously (9). Between December 1981 and September 1982, BEP was replaced by EP for stage II, III, and IV disease, but not for relapses. Details of both patient populations are shown in tables 1 and 2.

RESULTS

Chemotherapy

Etoposide and Cisplatin

Of 16 evaluable patients, 11 (69%) achieved complete remission with chemotherapy and an additional patient had a residual mass resected which showed fibrosis only. Thus, 12 of 16 patients (75%) achieved complete remission without any additional chemotherapy. One further patient failed primary treatment with EP but has been rendered disease-free with PVB and surgery. Patients have been followed from 13 to 23 months (mean, 18.9; median, 18.5) after the start of chemotherapy.

Table 1 shows details of the patient population. As shown, Patients 1-4 failed to achieve complete remission on EP. Patients 3 and 4 had increasing serum markers after three cycles of chemotherapy and were switched to PVB. Patient 3 is unusual in that metastatic disease not apparent on initial staging has been confined to the liver. Despite resection of a right hepatic metastasis and further chemotherapy, his disease remains uncontrolled. Patient 4 received PVB and then an abdominal mass was resected which showed differentiated teratoma. Two months later a mediastinal mass was excised which also showed differentiated teratoma. The patient is alive and in complete remission 15 months after starting EP and 8 months after the second surgical procedure.

Bleomycin, Etoposide, and Cisplatin

Of 18 patients, 16 (89%) achieved complete clinical remission, with normalization of serum markers. In two patients who had residual masses, surgery revealed mature teratoma. Thus, all 18 patients have been continuously disease-free since therapy, with observation times of 12-34 months (mean, 24.5; median, 25). Table 2 shows details of stage distribution, histology, chemotherapy, radiotherapy, and surgery.

TABLE 1.—EP for patients with small-volume metastases of testicular nonseminoma (The Royal Marsden Hospital, 1981–1982)

Patient No.	Stage	Histology*	No. of cycles of EP	Response to EP: Marker complete remission	Response to EP: Metastasis complete remission	Surgery after EP (histology)	Further chemotherapy†	Present status (mos)‡
1	$IVOL_1$	MTU/YS	4	Yes	No	Yes (MTU)	PVB, doxorubicin, methotrexate, dactinomycin	Alive with disease (18)
2	IIA	MTI/YS/sem	4	Not raised	No	No	Lung metastasis; JEB, methotrexate	Alive with disease (19)
3	IM	MTU/YS/sem	3	No	—	No	Relapse in liver; PVB, methotrexate, VAC, dactinomycin	Alive with disease (16)
4	IIB	MTI	3	No	No	No	PVB + surgery [differentiated (mature) teratoma]	NED (15)
5	IIA	MTU/YS	4	Yes	Yes	No	—	NED (21)
6	IIB	MTU	4	Yes	Yes	No	—	NED (17)
7	IIA	MTI/YS	4	Not raised	Yes	No	—	NED (22)
8	IIA	MTI	4	Not raised	Yes	No	—	NED (21)
9	IM	MTI/YS/sem	4	Yes	—	No	—	NED (21)
10	IIB	MTU	4	Yes	Yes	No	—	NED (16)
11	IIB	MTU	4	Yes	Yes	No	—	NED (22)
12	I§	MTU	4	Not raised	Not evaluable	No	—	NED (13)
13	IIB	Sem/AFP+	4	Yes	No	Yes (fibrosis)	—	NED (23)
14	IIB	MTU/YS/sem	4	Yes	Yes	No	—	NED (23)
15	$IVBL_1$	MTU	4	Yes	Yes	No	—	NED (16)
16	IIB	MTU/YS	4	Yes	Yes	No	—	NED (18)
17	IIA	MTT/YS	4	Yes	Yes	No	—	NED (13)

* MTU = undifferentiated malignant teratoma; YS = yolk sac carcinoma; MTI = intermediate malignant teratoma; sem = seminoma; and MTT = trophoblastic malignant teratoma.
†JEB = carboplatin, etoposide, and bleomycin; and VAC = vincristine, dactinomycin, and cyclophosphamide.
‡NED = no evidence of disease.
§Extension of tumor through tunica.

TABLE 2.—BEP for patients with small-volume metastases of testicular nonseminoma
(The Royal Marsden Hospital, 1981-1982)

Patient No.	Stage	Histology	No. of cycles of BEP	No. of cycles with 5 days of etoposide	Post-chemotherapy radiotherapy	Histology of excised residual masses*	Present status (mos from chemotherapy)†
1	IM	MTU/sem	4				NED (29)
2	IM	MTI	4			TD	NED (27)
3	IM	MTI	4	2			NED (31)
4	IIA	MTU	2		+		NED (34)
5	IIA	MTU	4				NED (12)
6	IIA	MTU	4				NED (25)
7	IIB	MTI	4	4	+		NED (33)
8	IIB	MTI/YS	4		+		NED (34)
9	IIB	MTT/sem	4	1	+		NED (30)
10	IIB	MTI	4			TD	NED (24)
11	IIB	MTI	4				NED (24)
12	IIB	MTU	4				NED (25)
13	IIB	MTI	4				NED (19)
14	IIIB	MTT	4				NED (22)
15	IVO/AL_1	MTU/YS	4				NED (28)
16	IVO/AL_1	MTI	4				NED (12)
17	IVO/AL_1	MTI	4				NED (19)
18	IVO/AL_1	MTI	4				NED (14)

*TD = differentiated (mature) teratoma.
†NED = no evidence of disease.

Interval Between Treatment Cycles and Outcome of Treatment

In the patients receiving EP, 18 of 49 (37%) intercycle intervals were prolonged from 3 to 4 weeks compared with 26 of 52 (50%) in the patients receiving BEP. Of five patients in the EP series, in whom the gap between the first and second cycles was 4 weeks, three failed to achieve complete remission compared with one of 11 where the gap was 3 weeks. In the patients receiving BEP, ten had a first gap of 4 weeks but none failed.

Toxicity (table 3)

Etoposide and Cisplatin

In EP-treated patients, platelet count nadirs ranged from 90,000 to 244,000/mm^3 but in only one patient was the count < 100,000/mm^3. Wbc count nadirs ranged from 1.4 to 4.3 × 10^3/mm^3, with 11 of 17 patients < 2.5, five of 17 < 2.0, and one of 17 < 1.5 × 10^3/mm^3. Hemoglobin levels fell to 8.7-12.1 g/L, with four of 17 patients having postchemotherapy levels of < 10 g/L. There were no instances of neutropenic fever or septicemia.

All patients had reversible alopecia and nausea and vomiting associated with cisplatin.

Renal clearance (^{51}Cr-EDTA) values were reduced by 0%-30% of pretreatment values (mean, 18%). By the end of chemotherapy, seven of 17 patients had clearances < 100, four of 17 < 90, one of 17 < 80, and nine of 17 < 70 ml/minute.

Impairment of lung function, Raynaud's phenomenon, and finger thickening or tenderness were not observed in the EP series. Two patients had minor numbness of fingers and toes and one patient developed classical but transient Lhermitte's syndrome. Four patients developed postchemotherapy gynecomastia.

Bleomycin, Etoposide, and Cisplatin

The most striking toxicity difference between EP and BEP was associated with the expected sequelae of bleomycin, with seven of 18 patients receiving BEP developing tender-thickened fingers, six of 18 developing tingling and numbness of fingers and toes, three of 18 developing Raynaud's phenomenon, and two of 18 developing symptomatic lung damage.

Platelet count nadirs ranged from 17,000 to 202,000/mm^3, with two of 18 patients < 100,000/mm^3. Wbc count nadirs ranged from 0.2 to 3.0 × 10^3/mm^3,

TABLE 3.—EP ± bleomycin for patients with small-volume metastases of testicular nonseminoma: influence of drug combinations on toxicity (The Royal Marsden Hospital, 1981–1982)

Toxicity	EP*		BEP†	
	No. of patients	%	No. of patients	%
Platelet count nadir < 100,000/mm³	1	6	2	11
Wbc count nadir < 2000/mm³	5	29	6	33
Hemoglobin nadir < 10 g/L	4	24	2	11
Fever treated with antibiotics	0	0	2	11
Renal clearance < 80 ml/min	1	6	0	0
Tenderness and thickening of fingers	0	0	7	39
Paresthesias/numbness in fingers/toes	2	12	6	33
Raynaud's phenomenon	0	0	3	17
Symptomatic lung damage	0	0	2	11
Postchemotherapy gynecomastia	4	24	6	33
Lhermitte's syndrome	1	6	0	0

* 49 cycles (37%) delayed by 1 wk.
†52 cycles (50%) delayed by 1 wk.

with ten of 18 < 2.5, six of 18 < 2.0, and three of 18 < 1.5 × 10^3/mm³. The lowest hemoglobin levels were 13.2–8.8 g/L, with two of 18 patients having postchemotherapy levels of < 10 g/L. Two of 18 patients experienced febrile episodes requiring antibiotics. None had proven septicemia.

DISCUSSION

The objective of investigating the EP combination was to develop an effective treatment for patients with testicular nonseminoma with limited-volume metastases, while reducing the toxicity associated with the use of bleomycin. Although hematological toxicity was comparable with that seen using the BEP regimen, other side effects, particularly lung toxicity, Raynaud's syndrome, and tenderness and stiffness of the hands, were avoided. It is of interest that one example of classical Lhermitte's syndrome was encountered in a patient receiving EP. This has been observed previously and is attributed to cisplatin.

After failure to achieve complete remission was documented in four of 16 evaluable patients, the EP study was discontinued, since it appeared that the two-drug combination was less effective than BEP. With the latter combination, all 18 patients with comparable stages of disease are alive and disease-free ($P = 0.07$) (table 1).

In the present small series treated with EP the data suggest that the omission of bleomycin might render the time interval between initial chemotherapy cycles more critical than is the case for BEP where, despite delays of 1 week (from 3 to 4), therapeutic outcome was not adversely affected (8). The finding in the present study that a 4-week gap between Cycles 1 and 2 was associated with eventual treatment failure in three of five patients compared with one of 11 patients in whom the interval was 3 weeks may be fortuitous. On the other hand, the difficulty of salvaging those patients who did not achieve complete remission with EP, despite small-volume disease, is compatible with the hypothesis that drug resistance is acquired extremely rapidly by germ cell testicular tumors and that intercycle intervals may be critically important.

It cannot be proved that the patients in the EP study who failed to achieve complete remission were not inherently chemotherapy-resistant from the outset, although from our previous experience with BEP in patients with small-volume metastases this seems unlikely. The acquisition of drug resistance during treatment is consistent with general experience of the difficulty of curing patients who have received prior chemotherapy, even where bleomycin-containing combinations are used as salvage therapy. This mechanism may explain the failure to cure patients with advanced bulky disease, where a poor vascular supply may limit drug access and exposure of clonogenic tumor cells to low concentrations of drugs could favor the development of resistance.

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Phase I Study of *N*-Methylformamide in Patients With Advanced Cancer[1,2,3]

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***N*-Methylformamide (N-MF) belongs to a class of polar-planar compounds which induce cellular differentiation. Preclinical antitumor activity was demonstrated against human mammary, colon, and lung tumor xenografts and L1210 and P388 murine leukemias. This phase I study used a single bolus infusion of N-MF given weekly × 3 doses every 6 weeks. Thirty-five patients were treated with N-MF at doses which ranged from 125 to 3125 mg/m²/week. The dose-limiting toxic effects included nausea and vomiting, anorexia, malaise, and liver function abnormalities. No myelosuppression was seen. The recommended dose for phase II trials of N-MF with this schedule is to initiate therapy at 2000 mg/m² weekly × 3 and escalate to 2500 mg/m² if the initial dose was well tolerated. [Cancer Treat Rep 69:489–493, 1985]**

N-Methylformamide (N-MF, NSC-3051) belongs to a class of polar-planar compounds which induce differentiation of specific cell lines in vitro (1,2). It is hypothesized that the drug acts by changing the conformation of DNA or DNA-protein complex, causing an alteration in transcription of the gene or genes that regulate expression of cellular differentiation.

N-MF was first evaluated in cancer patients at Memorial Sloan-Kettering Institute in 1956 because of the antitumor activity demonstrated in animals (3–5). Only five patients were treated with oral N-MF, but treatment was soon discontinued due to the hepatic toxicity seen (6).

More recently, Dexter et al (7) reported that *N,N*-dimethylformamide (DMF) caused a loss of tumorigenicity in cultured human colon carcinoma cells. This prompted the National Cancer Institute to evaluate DMF and its metabolite N-MF against a panel of tumors including human tumors.

Good antitumor activity was demonstrated by N-MF against the human MX-1 mammary, CX-1 colon, and LX-1 lung tumor xenografts implanted beneath the renal capsule of nude mice and against established sc implanted human mammary tumor xenografts. The drug has shown moderate activity against the ip implanted L1210 and P388 murine leukemias.

Preclinical toxicology studies were carried out by the Southern Research Institute using the CDF1 mouse and the beagle dog, using the iv route of administration. N-MF produced reversible toxicity to the hematopoietic system, characterized by leukocytosis (neutrophilia) and mild thrombocytopenia, and toxicity to the liver, characterized by increased liver enzymes and elevated total bilirubins (8). The latter toxicity was considered to be dose-limiting.

A phase I study of N-MF in patients with advanced cancer was initiated because of the drug's antitumor activity, as well as its lack of myelosuppression.

MATERIALS AND METHODS

The objectives of this study were to (*a*) determine the highest safely tolerable dose of N-MF administered iv weekly × 3 doses every 6 weeks, (*b*) describe and quantitate the toxicity of N-MF, and (*c*) seek preliminary evidence of therapeutic activity of N-MF in patients with advanced cancer.

Requirements for patient entry into the study included a histologic or cytologic diagnosis of cancer refractory to conventional therapeutic modalities, an Eastern Cooperative Oncology Group (ECOG) performance status of 3 or less, no major surgery within 14 days, no large field radiotherapy or chemotherapy within the last 28 days (42 days if the last chemotherapy included mitomycin or

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nitrosoureas), adequate bone marrow function (wbc count > 4000/mm^3, platelet count > 100,000/mm^3), hepatic function (bilirubin < 2.0 mg/100 ml), and renal function (creatinine < 1.5 mg/100 ml). The following parameters were evaluated prior to entry into the study: history and physical examination, weight, performance status, tumor measurement, cbc (including a differential), electrolytes, renal and liver function studies, glucose, uric acid, calcium, phosphorus, prothrombin time, urinalysis, chest x-ray, and electrocardiogram.

Patients were seen weekly during the time they were on study, and had pertinent history, physical examination, weight, hematology and chemistry studies, prothrombin time, and urinalysis repeated on each visit. Other studies were repeated as clinically indicated. Patients were continued on study until the development of progressive disease, the occurrence of unacceptable toxicity, or they requested that therapy with N-MF be discontinued.

Based on the preclinical toxicology data, the initial dose of N-MF administered in this study was one-thirtieth of the LD10 in the mouse or 125 mg/m^2. Dose escalations were made utilizing the modified Fibonacci search method. At least three patients were entered at each escalated dosage level. Dose escalation was permitted in patients who received a lower dose of the drug with no observable toxicity. As the maximally tolerated dose was approached and potentially dose-limiting toxicity was observed, at least six patients were entered at each dose level where toxicity that did not preclude further escalation was encountered. Patients were considered evaluable for toxicity if they completed the 6-week cycle of therapy or if they were removed from the study prior to receiving all three doses of N-MF because of drug toxicity. Patients were considered evaluable for response if they completed the 6-week cycle of therapy.

N-MF was supplied in ampules (2 ml per ampule), 400 mg N-MF/ml (40%) in 0.9% sodium chloride injection. Undiluted N-MF is an organic solvent. Concentrations of ⩽ 40% in contact with plastic administration sets do not leech appreciable amounts of the plasticizer diethylhexylphthalate. Since the compatibility of N-MF (40%) with other plastic items had not been determined at the time the study was initiated, glass syringes and glass infusion bottles were used for the preparation and administration of the N-MF. The drug was infused into the patient in 5–15 minutes.

RESULTS

Thirty-five patients (14 males and 21 females) were entered in the study. Patient characteristics are shown in table 1. The age of patients ranged from 28 to 74 years, with a median of 57 years. All patients except one had prior radiotherapy and/or chemotherapy. All patients treated had advanced solid tumors.

TABLE 1.—Patient characteristics

Characteristic	No. of patients
Sex	
Male	14
Female	21
Median age in yrs (range): 57 (28–74)	
Median performance status (range): 2 (0–3)	
Prior therapy	
Chemotherapy only	14
Radiotherapy only	1
Both	19
None	1
Site of malignancy	
Colon	9
Breast	4
Cervix	4
Ovary	4
Head and neck	3
Lung	3
Kidney	3
Melanoma	2
Sarcoma	2
Vagina	1

The 35 patients received 47 courses of drug therapy, of which 42 courses were evaluable for toxicity through ten dose escalations (table 2). Standard ECOG criteria for solid tumor toxicity and response were used (9). No antitumor responses were observed in any of the patients receiving N-MF. No toxicity was seen at doses < 1500 mg/m^2 weekly × 3.

Nonhepatic Toxicity

Toxic effects other than those related to liver function test abnormalities are shown in table 3. At 1500 mg/m^2, nausea and vomiting, anorexia, and malaise were observed. Nausea alone (grade 1) occurred in two of five courses, and vomiting, controllable with antiemetics (grade 2), occurred in three of five courses. These symptoms usually occurred several hours after drug adminis-

TABLE 2.—Dose escalations

Weekly dose (mg/m^2)	No. of patients (evaluable)	No. of courses (evaluable)
125	3 (3)	3 (3)
250	3 (3)	3 (3)
413	4 (4)	4 (4)
625	3 (3)	3 (3)
875	—	—
1125	4 (3)	4 (3)
1500	4 (4)	5 (5)
2000	11 (9)	14 (12)
2500	8 (6)	9 (7)
3125	2 (2)	2 (2)

TABLE 3.—N-MF toxic effects

Dose (mg/m^2)	No. of courses	Toxicity grade	Toxic effect*			
			Nausea and vomiting	Anorexia	Malaise	Diarrhea
1500	5	0	0	1	1	5
		1	2	2	2	0
		2	3	2	2	0
		3	0	0	0	0
2000	12	0	0	1	0	10
		1	3	5	2	2
		2	8	4	6	0
		3	1	2	4	0
2500	7	0	2	0	0	7
		1	1	5	5	0
		2	3	2	1	0
		3	1	0	1	0
3125	2	0	0	0	0	2
		1	0	0	0	0
		2	0	0	0	0
		3	2	2	2	0

* Values = No. of courses.

tration and lasted 12–48 hours. Malaise and anorexia were graded from 0 to 3, with 0 reflecting no symptoms and 1, 2, and 3 reflecting either mild, moderate, or severe symptomatology. Anorexia and malaise lasted longer than nausea and vomiting. Malaise and anorexia typically had their onset within 24 hours of drug administration and lasted for several days. With each successive dose in a course of therapy, the symptoms tended to become more pronounced and last for a more protracted period. These toxic effects, although subjective in nature and difficult to quantitate accurately, are of great importance, since they represent a major component of the toxicity of this compound. No diarrhea was noted. In three courses, grade 1 paresthesias of the fingertips and/or toes intermittently occurred.

At 2000 mg/m^2, grade 2 nausea and vomiting, anorexia, and malaise were observed, as well as the first episodes of grade 3 toxicity. Intractable nausea and vomiting was seen in one of the 12 courses administered. Severe anorexia (grade 3) was noted in two of 12 courses and severe malaise (grade 3) was noted in four of 12 courses. One patient receiving two weekly doses of 2000 mg/m^2 experienced severe chest pain associated with dyspnea, malaise, and nausea and vomiting 36 hours after each drug administration. The patient with each episode was hospitalized and treated symptomatically, with relief of symptoms after several hours. Pulmonary and cardiac causes of the symptoms were ruled out. The patient did not receive the third dose of N-MF.

At 2500 mg/m^2, both grade 2 and 3 nonhepatic toxic effects were seen. One patient had protracted vomiting for 96 hours after the first dose of N-MF and refused further therapy. One patient had an Antabuse-like reaction to alcohol following the first dose of N-MF. Another patient experienced grade 2 paresthesias of the soles of his feet occurring on Days 15 and 29 of the drug cycle.

Two patients received 3125 mg/m^2 of N-MF. Each patient received only the first dose of therapy, since both patients developed intractable vomiting which lasted for at least 8 days. Grade 3 anorexia and malaise were associated with this vomiting. No additional patients were given this dose level.

There was no treatment-related myelosuppression. A transient leukocytosis was seen at higher dose levels; however, this was not a consistent finding.

Hepatic Toxicity

Table 4 demonstrates the extent of the hepatotoxicity observed with this schedule of drug administration. The incidence and characteristics of the hepatic toxicity were variable at the 1500-, 2000-, and 2500-mg/m^2 dose levels. Using this grading system, grade 1 hepatotoxicity includes elevations of SGOT, SGPT, alkaline phosphatase, or bilirubin from 1.5 to 2 times normal. Grade 2 and 3 toxicity includes elevations from two to five times normal and greater than five times normal, respectively.

TABLE 4.—Hepatic toxicity

Dose (mg/m^2)	No. of courses	No. of courses with grade—			
		0	1	2	3
1500	5	1	2	1	1
2000	10	4	0	5	1
2500	6	3	0	2	1
3125	2*	1	0	0	1

* Only 1 dose of drug given.

Typically, elevations of alkaline phosphatase and/or liver transaminases occurred during the third to fourth week of the 6-week cycle, with normalization in most instances over the ensuing 2-week period. In 11 courses with grade 2 or 3 hepatic toxicity, an elevated bilirubin due to drug was seen in only three, one at 2000 mg/m^2 and two at 2500 mg/m^2. The highest bilirubin recorded was 5.4 mg/100 ml at 2000 mg/m^2.

Grade 2 and 3 toxicity occurred in two of five evaluable courses at 1500 mg/m^2, six of ten courses at 2000 mg/m^2, three of six courses at 2500 mg/m^2, and one of two patients treated with a single dose of 3125 mg/m^2. No clear increase in the incidence or severity of the hepatic toxicity could be demonstrated in this dose range. Only three of 12 patients receiving 14 courses of N-MF associated with hepatotoxicity were followed with further therapy with the drug, so little information is available from this trial about cumulative hepatotoxic effects. Table 5 demonstrates representative liver function test abnormalities from four courses delivered at the four highest dosage levels.

DISCUSSION

N-MF is a drug of considerable interest, mainly because of the class of compound to which it belongs and the unique side effects associated with its administration. N-MF belongs to a class of polar-planar compounds which induce cellular differentiation. It is a drug that produces no myelosuppression, but does cause reversible hepatotoxicity.

The major aims of this study were to establish the maximally tolerated dose of N-MF administered as a single iv bolus weekly × 3 every 6 weeks, and to recommend a dose for phase II studies of the drug. No toxicity was seen at doses < 1500 mg/m^2 weekly × 3. However, the hepatic and extrahepatic toxic effects of this drug noted at 1500, 2000, and 2500 mg/m^2 are qualitatively similar and make a firm recommendation of a phase II dose somewhat difficult, despite the fact that 19 evaluable patients have been treated at these dosage levels. This is due, in part, to the fact that the anorexia and malaise which represented the most consistent and severe toxic effects of this drug are poorly quantitated and also constitute a major symptom of progressive systemic malignancy. The incidence and severity of hepatic toxicity across those dose levels remained relatively constant and do not define the maximum tolerated dose or a recommended phase II dose. Our study does not fully address the question of whether cumulative hepatotoxicity might occur. Based on this experience, our recommendation for the phase II evaluation of N-MF in this schedule would be to initiate therapy at 2000 mg/m^2 weekly × 3 and escalate to 2500 mg/m^2 if the initial dose was well tolerated. Since the constitutional and hepatic toxicity encountered in our study did not appear to differ significantly based on pretreatment performance status or prior therapy, we cannot recommend different starting doses for so-called "good-risk" and "poor-risk" patients.

The nausea and vomiting encountered were mild to moderate in severity and were generally managed with standard antiemetics. Since prophylactic antiemetics were not employed in this study, no comment is possible on their efficacy. Other toxic effects encountered included alcohol intolerance in one patient (10), chest pain and shortness of breath temporarily associated with drug administration on two occasions in one, and numbness and tingling in the distribution of peripheral nerves in four.

In a phase I study conducted in The Netherlands utilizing a daily × 5 schedule of N-MF, McVie et al (11)

TABLE 5.—Representative examples of liver function abnormalities

Dose (mg/m^2)	Patient No.	Liver function test	Day 1	8	15	22	29	36	43
1500	014	SGOT (N = 0–41 IU/L)	23	24	52	183	83	87	27
		SGPT (N = 0–33 IU/L)	27	24	127	> 260	136	55	—
		Alkaline phosphatase (N = 37–108 IU/L)	67	51	76	149	51	44	73
		Total bilirubin (N = 0.3–1.2 mg/dl)	0.2	0.4	0.7	1.2	0.7	0.5	0.3
2000	018	SGOT	17	24	20	25	17	13	15
		SGPT	10	11	9	16	5	9	5
		Alkaline phosphatase	79	162	156	281	130	118	119
		Total bilirubin	0.2	0.5	0.5	0.8	0.4	0.3	0.2
2500	024	SGOT	45	49	87	—	109	100	93
		SGPT	37	41	48	—	53	41	36
		Alkaline phosphatase	316	400	609	—	671	573	501
		Total bilirubin	0.4	0.6	0.4	2.4	0.7	1.0	0.8
3125	029	SGOT	20	> 400	18	16	—	20	—
		SGPT	11	> 260	64	23	—	7	—
		Alkaline phosphatase	76	174	96	83	—	—	—
		Total bilirubin	0.2	1.0	0.4	0.3	—	0.2	—

reported the occurrence of hyperbilirubinemia in nine of 18 cycles at 1000 mg/m^2 daily. At the maximum tolerated dose of N-MF in our study, 2000 mg/m^2 weekly × 3, hyperbilirubinemia occurred in one of ten courses. However, nausea and vomiting, anorexia, and malaise appeared more severe on the weekly × 3 schedule than on the daily × 5 schedule.

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Phase II Study of rDNA Human Alpha-2 Interferon in Multiple Myeloma[1]

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This is a report of a phase II trial of rDNA human alpha-2 interferon (IFN) (Schering-Plough 30500) in previously treated patients with multiple myeloma. Patients received 2 megaunits/m² of IFN by sc injection 3 days per week for 3 months. Responding patients continued receiving the same dose, but those with stable disease received 10 megaunits/m² three times per week. Eighteen patients were treated. Two partial responses were seen, one at the lower dose and one at the higher dose. The lower dose was well tolerated, but three of six patients who received 10 megaunits/m² had to stop treatment because of subjective toxicity. Hematological toxicity was mild. At this dose and schedule, IFN has only minimal activity in previously treated patients. [Cancer Treat Rep 69:495–498, 1985]

Several studies have suggested that interferon (IFN) may be active in the treatment of multiple myeloma (MM) (1–5). Because these early studies used leukocyte IFN, which contains a mixture of alpha IFN, there have been difficulties with the formulation and purity and thus with objective evaluation of the data (4). New developments in molecular biology have meant that highly pure single species of human IFN are available for clinical trial for the first time. Phase I studies of these agents have been published (6–8). This article reports the results of a phase II study of rDNA human alpha-2 IFN in patients with MM.

MATERIALS AND METHODS

This was a single center study designed to evaluate the efficacy of rDNA human alpha-2 IFN (Schering-Plough 30500) in patients with MM who had relapsed during or after conventional chemotherapy. IFN was provided by the Schering-Plough Corporation. Formulated alpha-2 IFN dissolved in phosphate-buffered saline (pH 7.2) was freeze-dried in sterile vials and stored at 4°C. It was reconstituted immediately prior to injection by adding 1 ml of sterile pyrogen-free water.

A dose of 2 megaunits/m² was administered sc 3 days per week (Monday, Wednesday, and Friday). The first injection was given in the hospital, and the patient was observed overnight. If no untoward reaction occurred, the rest of the treatment was given either by the community nurse or by the patients themselves on an outpatient basis. For the first month, the patients were seen at 2-week intervals, but subsequently only monthly. Evaluation of toxicity and response was made at these time points.

Treatment was continued for 3 months, provided that progressive disease, as defined below, did not occur. If the patient was responding at the end of that time, IFN was continued either until there was progressive disease or to 12 months of treatment. If the disease was stable at 3 months, the IFN dose was escalated to 10 megaunits/m² three times per week. Treatment was continued at this higher dose for a further 3 months, with stable or responding patients continuing for up to 12 months after that time.

Partial response (PR) was defined as a decrease of $\geq 50\%$ in the serum paraprotein levels and the urinary light chain excretion, provided that the pretreatment value of the latter was > 1.0 g/24 hours (9). For a response to be recorded, the above criteria must have been maintained for at least 4 weeks. Progressive disease was defined as an increase of $\geq 25\%$ in either the serum paraprotein level or the 24-hour urinary light chain excretion. Stable disease was defined as between a -50% and $+25\%$ change in either the serum paraprotein level or 24-hour urinary light chain excretion.

A full biochemical screen, including assessment of renal function, myeloma status, beta-2 microglobulin, and serum calcium, was performed prior to entry in the study and then at each hospital visit. A skeletal survey,

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bone marrow aspirate, and trephine biopsy were performed before therapy and then at 3-month intervals, unless progressive disease was thought to have occurred.

Patients were considered eligible if they had a diagnosis of MM, as defined by the Myeloma Task Force of the National Cancer Institute (9). Patients had to have progressive disease as defined above, and had to have relapsed while receiving or after receiving conventional chemotherapy. Patients should not have received chemotherapy for at least 4 weeks (6 weeks for nitrosoureas and melphalan) and should not have been treated with extensive radiotherapy during the same period. The Karnofsky performance status had to be ⩾ 50% and life expectancy had to be at least 4 months. A previous diagnosis of another malignancy or prior therapy with IFN excluded patients from the study.

The characteristics of the 18 patients entered into the study are shown in table 1. One patient (No. 14) failed to receive therapy for 1 month. He died of bronchopneumonia after receiving only three injections (1 week) of IFN. Three other patients (Nos. 10, 15, and 18) received IFN for only 1 month. These patients all had rapidly progressive disease associated with deterioration in their general condition.

There were no complete responders in this study. Two patients achieved PR lasting 12 (No. 5) and 16 (No. 6) weeks, according to the criteria stated above. Patient 5 had kappa light chain disease assessable only by urinary light chain excretion. Her light chain excretion fell by 64% after 1 month of IFN (ie, PR), but interpretation of this response is complicated by the presence of renal impairment. Her glomerular filtration rate (GFR), serum urea, and creatinine remained stable during the first 2 months of treatment with IFN when the urinary light chain excretion was falling (1645–445 mg/24 hours). However, during the third month of IFN, the GFR fell by 50%, and the patient eventually died of renal failure 36 days after stopping IFN. The second patient with an IgG kappa myeloma, who qualified as a PR (No. 6), had stable disease after 3 months of IFN at 2 megaunits/m^2, but responded at the higher dose of 10 megaunits/m^2 after 1 month of therapy. Both of these patients had extensive lytic bone disease which did not show evidence of healing at the time of their biochemical response.

Three other patients (Nos. 1, 8, and 12) had decreases ⩾ 50% in their GFR, but these were not maintained for 4 weeks. In all, eight patients, other than those who achieved PR, had decreases of 25%–50% in either their serum paraprotein level or urinary light chain excretion during the first 2 months of therapy, but these decreases were not maintained and thus PR was never achieved.

Serum beta-2 microglobulin (B2M) was measured serially in all patients, since this has been suggested to be a value in assessing response in MM (10). None of the patients showed sustained decreases indicative of response.

Eleven patients stopped the lower dose of IFN because of progressive disease. No patients at this dose stopped therapy because of toxicity. In six patients, IFN was escalated to 10 megaunits/m^2. Three of these patients stopped treatment with IFN because of subjective toxicity (Nos. 7, 9, and 12) consisting of malaise, tiredness, lethargy, and influenza symptoms, without having evidence of progressive disease. One of these patients (No. 11) stopped treatment with IFN because of progressive disease; one (No. 8) died suddenly; and the final patient (No. 6) achieved a PR and continued to receive IFN for a full 12 months.

Hematological toxicity was difficult to evaluate objectively due to the presence of heavy bone marrow involvement by MM in all of the patients. Changes in the blood cell counts are shown in table 2. There is a suggestion from these data that for the wbc count, the World Health Organization (WHO) grade increases by one as a result of treatment with IFN at 2 megaunits/m^2. Platelet counts are affected less. Two patients (Nos. 3 and 13) developed severe hematological toxic effects (WHO grade 4 for both wbc and platelet counts), which recovered by 1 month after stopping treatment with IFN. Three patients developed severe life-threatening infections. Two of these patients (Nos. 8 and 14) died, and the third (No. 3) recovered. There were no episodes of life-threatening hemorrhage.

Five patients died during or soon after this study. Two patients died as a result of infection: the first (No. 14) developed bronchopneumonia after only three injections of IFN, and the second (No. 8) received 2 megaunits/m^2 of IFN for 4 months and then 10 megaunits/m^2 of IFN for 6 months. This patient died suddenly of gram-negative septicemia. On the last admission to the hospital, her wbc count was 2.4×10^9/L. Three other patients (Nos. 10, 15, and 18) developed rapidly progressive MM, which was responsible for their death. These patients died 7, 12, and 44 days after stopping IFN, respectively.

DISCUSSION

Although two of 18 patients achieved PR by objective criteria, these responses were of short duration. Therefore, the response rate is 11%, with 95% confidence limits being between 0% and 26%. The interpretation of the data from one of these responding patients with light chain disease is complicated by the presence of renal impairment. Reduced glomerular filtration will decrease urinary light chain excretion, but a tubular defect might increase it, since light chain is metabolized by renal tubular cells (11). This patient did not have a serum paraprotein, and the serum B2M levels probably rose as a consequence of renal impairment, although possibly because of progressive MM. Both of these responding patients had relapsed after prior chemotherapy rather than during it. This may indicate that they were not truly refractory to conventional chemotherapy. Neither patient has received

TABLE 1.—Characteristics of patients entered into a phase II study of rDNA human α_2 interferon

Patient No.	Sex	Age (yrs)	Myeloma type	Stage	Performance status at entry (%)	Time from diagnosis (mos)	Prior therapy*	Response to prior therapy†	Relapse/progression during (D) or after (A) prior therapy
1	M	64	κ light chain disease	IIIA	70	89	X,MCP,A	SD (MCP) PD (A)	D
2	F	67	IgG κ	IIIA	60	15	X,MCP	PD	D
3	M	58	IgG κ	IIIA	90	33	X,MCP,C	PR	D
4	F	54	κ light chain disease	IIIB	60	41	X,MCP,VD	PR	D
5	F	58	κ light chain disease	IIIB	60	66	X,MCP	PR	A (10 mos)
6	F	35	IgG κ	IIIA	90	23	X,MCP,MP	SD	A (3 mos)
7	M	72	IgG λ	IIIA	60	32	X,MCP,C	SD (MCP) PD (C)	D
8	F	48	IgA λ	IIIA	80	46	X,MCP,MP	PR (MCP) SD (MP)	A (4 mos)
9	F	68	IgG κ	IIIA	60	23	X,MCP,MP	PR (MCP) PD (MP)	D
10	F	68	IgA κ	IIIA	60	22	X,MP,V	SD (MP) SD (V)	A (3 mos)
11	F	44	IgG λ	IIIA	80	51	X,MCP,AB	PR (MCP) PD (AB)	D
12	F	64	IgG κ	IIIA	80	81	X,MCP_1,A,MCP_2	PR ($MCP_{1\&2}$) SD (A)	A (16 mos)
13	M	60	IgA κ	IIIB	80	28	MP,AB	PR (MP) PD (AB)	D
14	M	71	IgG λ	IIIA	60	23	X,MCP	PD	D
15	M	60	IgA κ	IIIA	60	56	X,MCP	PR	D
16	M	54	IgG λ	IIIA	60	108	X,MCP,C,MP	All PR	D
17	M	60	IgG κ	IIIA	80	28	X,MCP	PR	D
18	M	57	IgD λ	IIIB	60	22	X,VMCP,AB	PD (VMCP) PR (AB)	D

*X = x-ray therapy; MCP = melphalan, cyclophosphamide, and prednisolone; A = cytarabine; C = cyclophosphamide; VD = vindesine; MP = melphalan and prednisolone; V = vincristine; AB = doxorubicin and carmustine; and VMCP = vincristine, melphalan, cyclophosphamide, and prednisolone.

†SD = stable disease; PD = progressive disease.

TABLE 2.—Blood cell count changes for patients with MM receiving 2 megaunits/m² of rDNA human α_2 IFN 3 times/wk

WHO grade	Cell count (× 10^9/L)	No. of patients				
		Pretherapy	1 mo	2 mos	3 mos	1 mo posttherapy
0	Wbc: ≥ 4.0	5	2	2	2	5
	Platelet: ≥ 100	14	9	8	8	8
1	Wbc: 3.0–3.9	6	6	4	3	0
	Platelet: 74–99	3	2	2	0	0
2	Wbc: 2.0–2.9	3	5	4	2	4
	Platelet: 50–74	1	3	2	1	0
3	Wbc: 1.0–1.9	3	3	4	2	2
	Platelet: 25–49	0	1	0	1	2
4	Wbc: < 1.0	1	1	0	1	0
	Platelet: < 25	0	2	2	0	1
Evaluable patients		18	17	14	10	11

further chemotherapy after stopping treatment with IFN. A further eight patients did have decreases in paraprotein levels, perhaps indicating an effect of IFN. This group of patients with minor decreases in paraprotein levels may show that IFN is effective in a higher proportion of patients with MM than the PR rates alone indicate. The decreases may not have reached PR because the patients entered into this study were all heavily pretreated and refractory to treatment.

At 2 megaunits/m² three times per week, IFN was well tolerated, but at 10 megaunits three times per week, it caused unacceptable toxicity in three of the six patients treated at this dose level. It is not clear from the available data whether there are dose-response or schedule effects for IFN in patients with MM. Further studies in this regard are required.

These data are not as encouraging as those reported with other IFNs (3–5) or with this IFN in different doses and schedules (12). The most recent results from the M. D. Anderson Hospital and Tumor Institute (4) showed that three of 12 (25%) previously untreated patients and two of nine previously treated patients responded. In the American Cancer Society study (5), three of 11 (27%) patients responded. The most recent update of the Swedish randomized trial gave a response rate of only 14% in 62 previously untreated patients with MM. These results were significantly inferior to those using melphalan and prednisone. Using alpha-2 IFN, Costanzi et al (12) have reported responses in seven of 38 (18%) largely heavily pretreated patients. Overall, of 112 patients treated by various types, doses, and schedules of IFN, there have been 19 responses (17%).

On the basis of these results, it seems that IFN alone is inferior to conventional chemotherapy for the treatment of MM. However, IFN does have sufficient activity to justify its inclusion in combination regimens. Indeed, there is experimental evidence suggesting that there may be synergy between cytotoxic agents and IFN (13,14). Studies to elucidate the role of IFN in combination with other agents are therefore necessary and are underway.

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Phase II Trial of Ifosfamide With Mesna in Previously Treated Metastatic Sarcoma[1]

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Ifosfamide given to 42 patients iv at 2–2.5 g/m²/day × 4 resulted in partial responses in ten of 28 (36%) evaluable patients with adult soft tissue sarcomas, including two of two with chondrosarcoma; none of nine with pediatric sarcomas (Ewing's sarcoma, osteogenic sarcoma, or rhabdomyosarcoma) achieved partial response. All of the pediatric patients had failed to respond to complicated three- to six-drug regimens of up to 18 months in duration. The response rates in patients with and without prior cyclophosphamide (32%; seven responses among 22 patients; and 20%, three responses among 15 patients) were not significantly different, supporting in vitro evidence of a lack of cross-resistance between the two related compounds. Myelosuppression was dose-limiting. Hemorrhagic cystitis was not observed in patients treated with 400–500 mg of mesna iv every 4 hours during ifosfamide treatment. Nausea and vomiting were generally mild or moderate. Alopecia was universal but reversible. Of the first 11 patients, five became somnolent or developed visual hallucinations (during six of the 12 total courses administered to the five patients). Only one patient had two episodes of neurotoxicity. After reduction of the use of iv antiemetics and major narcotics, single episodes of neurotoxicity were seen in five of the next 27 patients. An asymptomatic acidosis developed in most patients, requiring bicarbonate replacement in one. Ifosfamide appears to be active in previously treated patients with sarcomas and should be evaluated in patients with less extensive prior treatment. [Cancer Treat Rep 69:499–504, 1985]

Ifosfamide is an analog of cyclophosphamide, with one chloroethyl group on each nitrogen. Like cyclophosphamide, ifosfamide is activated by liver microsomes via hydroxylation of the No. 4 ring carbon (1). Incubation of liver microsomes with ifosfamide produces alkylating activity at half the rate of cyclophosphamide; thus, the dechloroethylation reaction which produces inactive metabolites is a more important metabolic pathway for ifosfamide. Because a smaller proportion of ifosfamide is activated, a larger dose is required. The higher concentration of metabolites increases the incidence of cystitis.

In tissue culture and in laboratory animals, ifosfamide is more active and less toxic than cyclophosphamide against Yoshida ascites sarcoma, Ridgway osteogenic sarcoma, and carcinosarcoma (2). Animal studies (3) and phase I and II trials in human lymphoma (4) and small cell lung cancer (5) have shown a lack of cross-resistance with cyclophosphamide, less myelosuppression, and more frequent hemorrhagic cystitis. Mesna has been reported to prevent hemorrhagic cystitis and to allow administration of larger doses of ifosfamide (6). Inactive as the dimesna form in the circulation, mesna is reactivated in the kidney. Thus, mesna does not appear to interfere with the antitumor effect of ifosfamide.

European investigators have reported response rates of 20%–78% for ifosfamide in pretreated sarcomas (table 1). We report the results of a phase II study in previously treated advanced sarcomas.

METHODS

Patients fulfilling the following criteria were eligible for participation: histologically confirmed metastatic sarcoma; measurable disease; performance score of 0–2 (< 50% of waking hours in bed); failure after a doxorubicin chemotherapeutic regimen; normal end-organ function (creatinine < 1.5 mg/dl; normal liver function tests); and no clinically evident congestive heart failure.

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TABLE 1.—Efficacy of ifosfamide in sarcomas in order of dose delivered/course

Investigator (Ref No.)	Ifosfamide dose (g/m^2) and schedule	Dose/course (g/m^2)	Other drug	Response* CR	Response* PR	No. of responses/ No. treated (%)	Sarcoma†
Klein et al (8)	2.5–3.5‡/day × 5	12.5–17.5		1	4	5/11 (45)	STS§
Czownicki and Utracka-Hutka (6,9)	1.25–2.5‡/day × 5	12.5		1	4	5/13 (38)	STS¶
Marti et al (10)	1.8‡/day × 5	9.0		2	4	6/18 (33)	OGS
Bierbaum et al (11)	1.65/day × 5	8.25	Cisplatin	3	5	8/16 (50)	STS
				1	0	1/4 (25)	OGS
				1	0	1/1 (100)	Ewing's
Stuart-Harris et al (12)	5 or 8 over 24 hrs q 3 wks	8.0		6	9	15/40 (38)	STS
Wellens et al (13,14)	1.5/day × 5	7.5	Etoposide	2	1	3/13 (23)	STS§
de Kraker and Voute (7)	3.0/day × 2 q 3 wks	6.0	Vincristine	2	5	7/9 (78)	STS
				2	1	3/4 (75)	OGS

* CR = complete response; and PR = partial response.
†STS = soft tissue sarcoma; OGS = osteogenic sarcoma; and Ewing's = Ewing's sarcoma.
‡ Calculated from mg/kg as dose in g/kg × 70/1.7 m^2 = g/m^2.
§ All CYVADIC (cyclophosphamide, vincristine, doxorubicin, and dacarbazine) failures.
¶ Three rhabdomyosarcomas: median survival for responders, 6 mos; for nonresponders, 3 mos.

Ifosfamide was supplied by the National Cancer Institute (Bethesda, MD). Mesna was provided by Asta Werke (Bielefeld, West Germany).

A urinalysis was performed daily before ifosfamide administration. If there were < 50 rbcs/μl of urine, ifosfamide (2.0 g/m^2) was infused iv over 4 hours daily for 4 consecutive days every 3 weeks. The dose was escalated to 2.5 g/m^2 if toxicity was acceptable at 2.0 g/m^2/day × 4.

To prevent hemorrhagic cystitis, patients received 300 ml/hour of iv fluid for 3 hours before and 200 ml/hour for 12 hours after ifosfamide. To offset any antidiuretic effects of ifosfamide, furosemide (20 mg iv) was administered as often as necessary if urine output dropped to < 200 ml/hour. The first four patients entered in the study were given their first course without mesna because it was unavailable. Thereafter, a dose of mesna equal to 20% of the ifosfamide dose was given 15 minutes prior to ifosfamide therapy and every 4 hours for five doses after each dose of ifosfamide.

Courses of ifosfamide were repeated every 21 days. Subsequent courses were delayed by 1 week if the wbc count had not returned to 3000/μl and if the platelet count had not returned to 100,000/μl. Drug was withheld for any significant neurologic abnormality or creatininemia > 2.0 mg/dl until the abnormality cleared.

Standard response criteria were used. PR was defined as a ≥ 50% reduction in the product of perpendicular diameters of all measurable lesions for > 4 weeks. Duration of response was calculated from the initiation of chemotherapy to the documentation of disease progression above measurements taken prior to entry.

RESULTS

Forty-three patients were entered in the study between November 27, 1983, and September 17, 1984 (table 2). Patients ranged in age from 13 to 71 years (median, 40), and all had failed a doxorubicin-containing regimen.

Six patients were not evaluable for response (table 2). Two early deaths were due to hepatorenal failure and respiratory failure in patients with large liver and pulmonary metastases, respectively. A third patient with stable disease after the first cycle refused further ifosfamide because of somnolence and visual hallucinations (*see* CNS toxicity). A fourth and fifth patient were both removed from the study on Day 3 when congestive heart failure developed. Sarcoma could not be established on histological review of the sixth patient.

Toxicity was predominantly myelosuppression. Patients received a median of 2.5 courses (range, one to more than eight). Days 10–14 blood cell counts were available for 86 of the 139 courses given. Most patients had wbc count nadirs between 1000 and 3000/μl. A wbc count nadir < 1000/μl developed after 23 courses; platelet count nadirs < 20,000/μl developed after six courses in three patients who had had extensive prior radiotherapy.

Hemorrhagic cystitis occurred in two of the four patients receiving ifosfamide without mesna. One patient subsequently tolerated escalated doses of ifosfamide with mesna without further hematuria. After mesna became available, no further gross hematuria was observed, although one patient had a history of bloody urine between cycles at the time of a documented urinary tract infection.

Because of the reported antidiuretic hormone-like effect of ifosfamide and the ~ 3 L/day of fluid given to prevent cystitis, furosemide (20 mg iv) was administered if urine output in any given 2-hour interval fell to < 200 ml/hour. Three patients developed pulmonary edema when this precaution was not scrupulously observed. In one patient ifosfamide was resumed without further incident after diuresis. The other two patients were removed from the study on Day 3 of their first course.

Five of the first 11 patients treated developed visual hallucinations or somnolence, which progressed in one patient to a state of unresponsiveness. These symptoms persisted up to 48 hours after discontinuing ifosfamide and mesna. Of a total of 12 courses administered to these five patients, six episodes of neurotoxicity were observed. No seizures were observed. All five patients were receiving iv antiemetics including perphenazine infusions, iv pentobarbital, and/or tetrahydrocannabinol (active ingredient in marijuana) and narcotic pain medications including hydromorphone or methadone. With care taken to minimize the use of iv antiemetics and major narcotics, excessive somnolence developed in only five of the next 27 patients. Two of these five patients refused further therapy. Three patients had a second course without developing neurotoxicity, presumably because other neurotoxic agents were minimized. These reversible neurologic abnormalities were encountered in patients 20–30 years of age as well as in older patients.

The degree of nausea and vomiting was highly variable but mild to moderate in most patients. Those patients with more severe vomiting often had had extensive prior chemotherapy and anticipatory nausea beginning several hours prior to receiving ifosfamide.

A previously unreported metabolic acidosis developed in most patients, with a progressive fall in serum bicarbonate levels to as low as 11 mEq/L. A similar acidosis has been observed during sodium thiosulfate administration to prevent cisplatin renal toxicity (15); thus, this acidosis may have been due to mesna or to forced hydration. In all patients, the acidosis resolved spontaneously and was clinically asymptomatic although bicarbonate replacement was required in one patient.

There were ten PRs (table 2). The median time to response was two cycles; however, one patient received five courses prior to achieving a PR. Ten of 28 patients (36%) with *adult* sarcomas responded, including both of the two with chondrosarcoma. Responses persisted 1–10+ months; the patient with the shortest response refused further ifosfamide after the second course. Seven of the responding patients had failed to respond to prior cyclophosphamide (table 3). The response rates for patients with and without prior cyclophosphamide (32%, seven responses among 22 patients; versus 20%, three responses among 15 patients) were not significantly different. None of the nine additional patients with pediatric sarcomas (Ewing's sarcoma, osteogenic sarcoma, or rhabdomyosarcoma) responded. However, all of these patients had relapsed after complicated four- to six-drug regimens of up to 18 months in duration.

DISCUSSION

About twice as many Americans die of sarcoma each year as of Hodgkin's disease, although the incidence of these two neoplasms in the United States is similar (7000 new cases/year) (16). The management of sarcoma, which frequently develops in young adults, has been complicated by a high rate of dissemination and resistance to cytotoxic treatment.

The objective response rate is 34% for the most active single agent, doxorubicin (17). Dacarbazine, cyclophosphamide, vincristine, and dactinomycin have some limited efficacy against adult soft tissue sarcomas (18–23), but the latter three agents are efficacious against pediatric sarcomas. The lack of additional active agents in adult soft tissue sarcoma has prevented the development of effective chemotherapy programs given as an adjuvant to primary surgery and radiation therapy.

In this study all patients had failed one or two prior chemotherapy regimens. Patients with osteogenic and Ewing's sarcomas and rhabdomyosarcoma had failed after up to 18 months of four- to six-drug chemotherapy regimens. Responses rates are frequently lower after prior chemotherapy, even for drugs with documented activity. For example, doxorubicin had a 14% response rate against sarcomas failing prior chemotherapy in an Eastern Cooperative Oncology Group phase II trial (24). Thus, the response rate for ifosfamide in untreated sarcomas may be significantly higher.

Toxicity

Hemorrhagic cystitis has been the major reported toxic effect, with an overall rate of 18%–40%. The severity can be diminished with mesna or *N*-acetyl cysteine, and to a lesser extent, bladder irrigation or ascorbic acid. Because of ifosfamide's relatively long half-life of 7–15 hours (25), bladder protection must be extended for prolonged periods. Cystitis may be exacerbated by the antidiuretic effect of ifosfamide, with a resulting fall in urine output and increased urine concentration of metabolites.

At daily doses of > 5 g/m^2 of ifosfamide, mental confusion, lethargy, somnolence, paresthesias, and disorientation have been observed in occasional patients (< 3%) in other studies, occurring predominantly in older patients (26,27). In the previously reported studies, symptoms began a few hours after infusion and persisted 1–5 days. Seizures had also been observed in one patient with brain metastases (26). Recovery has been complete in both previously reported and current patients. CNS toxicity may result from water intoxication secondary to antidiuretic hormones (26), but neither urine nor serum electrolytes

TABLE 2.—Patient characteristics: ifosfamide and mesna in advanced sarcoma, arranged by response and histology

Patient No.	Age (yrs)	Sex	Diagnosis*	Grade	Prior treatment†		Measurable disease	Highest dose/ course (g/m^2)	Response duration (mos)‡	Comments
					Radiation therapy	Chemotherapy				
1	52	F	MFH	3	Pelvis	D, C, Da	Abdomen, liver	8	PR (10+)	Abdominal mass, 12 × 16 to 4.5 × 4 cm
2	47	M	MFH	3	None	D, PII	Lung	8	PR (7)	Pulmonary metastasis reduced by 60%–100%
3	18	M	MFH	—	Leg	D, C, V, Da	Lung	8	PR (3+)	Pulmonary metastasis reduced by 50%
4	40	F	LMS	1	Pelvis	D, V, A, C, P, F	Abdomen	8	PR (6+)	Abdominal nodules gone at laparotomy
5	58	F	LMS	2	Sternum	D	Lung	10	PR (6)	Pulmonary metastasis reduced by > 95%
6	23	F	Cho	2-3	Chest	D, B, A, C	Neck	10	PR (1)	Neck mass: 6 × 6 to 4.5 × 4 cm
7	40	M	Cho	3	Chest	D	Lung	10	PR (7)	Lung mass: 16.5 × 11.5 to 12 × 7.7 cm
8	39	F	Fib	3	Chest	D, C, Da	Lung	8	PR (3+)	Skin and hilar mass reduced by > 50%
9	42	F	Epith	3	Chest	D, C, Da	Lung	8	PR (2+)	Pulmonary metastasis reduced by ,50%–100%
10	50	M	Neuro	3	Groin	D, C, V, Mc	Lung	8	PR (3)	Pulmonary metastasis reduced by 65%
11	53	F	LMS	3	Pelvis	D	Lung	10	MR (5)	Pulmonary metastasis reduced by 25%
12	54	F	LMS	3	None	D	Liver	10	MR (6)	LDH, 543 to 130
13	71	F	Ang	2	Chest	D, P	Skin	8	MR (2)	Skin lesions reduced by 25%
14	59	F	LMS	3	Abdomen	D	Lung	10	SD (5)	
15	40	F	LMS	3	None	D	Neck, lung	10	SD (6)	
16	49	F	LMS	1	CNS, retroperitoneum	D, C, V, Da	Pleura, liver	10	SD (4+)	
17	54	F	LMS	—	None	D, PII		10	SD (2)	
18	58	M	LMS	—	Hip, lung	D, Da	Lung	8	SD (3)	
19	58	F	MM	3	None	D, C	Peritoneum	8	SD (2)	
20	34	F	MM	2	None	D, C, Mc, P	Pleura	8	SD (7)	
21	53	M	MM	2-3	None	D, P, D, C	Lung	8	SD (2)	
22	64	F	MFH	2	Chest	D, V, PII	Lung	8	SD (3)	
23	13	M	RMS	3	Arm/foot	D, V, A, C	Lung, pleura	8	SD (5)	Decreased effusion
24	22	M	Neuro	—	None	D, C, Da		10	SD (5+)	
25	42	M	Spdl	—	Pelvis	D, C, V, M		10	SD (6+)	
26	19	F	OGS	3	None	D, P, C, V, M	Lung	8	SD (9)	
27	20	F	ES	3	Right humerus	V, A, C, D	Lung	8	DP	
28	19	M	ES	3	Pelvis	D, V, A, C, V, B, P	Abdomen, lung	6	DP	
29	24	M	ES	3	Left arm	D	Arterial mass	10	DP	
30	19	M	ES	3	Fibula	V, A, C, D	Bone	8	DP	

31	14	F	ES	3	Fibula	V, A, C		8	DP	
32	14	F	OGS	3	None	D, B, C, A, M, P	Lung	8	DP	
33	26	F	RMS	3	Neck, L3	D, V, A, C	Neck, bone	6	DP	
34	49	F	LMS	3	Left hip	D, P, Da	Lung	8	DP	
35	47	M	LMS	2-3	None	D, V, C		8	DP	
36	66	F	Neuro	3	Buttock	D		8	DP	
37	28	M	GCB	—	None	D, Da, M, PII	Lung	8	DP	
38	53	F	MFH	1	Lung	D, C, Da	Lung	3	NE	Off study on Day 3, congestive heart failure
39	70	M	MFH	2	Retroperitoneum	D, Da	Abdomen, liver	4.5	NE	Died on Day 11, liver failure
40	56	F	LIP	2-3	Retroperitoneum	D, C, Da	Lung	8	NE	Died on Day 17, respiratory failure
41	53	F	LMS	3	None	D	Retroperitoneum	8	NE	Refused further ifosfamide
42	67	M	MM	3	None	D, C	Lung	6	NE	Off study on Day 3, congestive heart failure
43	36	F	?	3	None	D, C	Lung	8	NE	Undifferentiated malignancy on review

* MFH = malignant fibrosis histiocytoma; LMS = leiomyosarcoma; Cho = chondrosarcoma; Fib = fibrosarcoma; Epith = epithelial sarcoma; Neuro = neurosarcoma; Ang = angiosarcoma; MM = mesothelioma; RMS = rhabdomyosarcoma; Spdl = spindle sarcoma; OGS = osteogenic sarcoma; ES = Ewing's sarcoma; GCB = giant cell sarcoma of bone; and LIP = liposarcoma.

† D = doxorubicin; C = cyclophosphamide; Da = dacarbazine; PII = phase II agent; V = vincristine; A = dactinomycin; P = cisplatin; F = 5-FU; B = bleomycin; Mc = mitomycin; and M = methotrexate.

‡ MR = minimal response; SD = stable disease; DP = disease progression; and NE = not evaluable.

TABLE 3.—Details of prior cyclophosphamide therapy in responding patients

Patient No.*	Dose (mg/m^2)	Cycles	Evidence of failure
1	400	q 3 wks, Feb-Aug 1983	Enlarged liver metastases
2	None	—	—
3	500	Feb 1983-Apr 1984	New pulmonary lesions
4	600	9	New nodules at laparotomy
5	None	—	—
6	600	2	Enlarged skin and neck masses
7	None	—	—
8	600	2	Progression in skin and lung
9	600	2	Progression in lung
10	500	2	Progression in lung

* Patients 1-10 from table 2.

suggested this as a factor in the current series. The high incidence of somnolence and visual hallucinations in five of our first ten patients was unexplained; however, only five of the next 27 patients developed CNS toxicity when the use of iv antiemetics and major narcotics was minimized.

Based on European studies (table 1) as well as the current series, ifosfamide appears active in advanced adult sarcoma and should be evaluated alone and in combination in previously untreated patients. Prior failure after cyclophosphamide therapy did not diminish the likelihood of a response to ifosfamide in the current study.

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Sister Chromatid Exchanges Induced by Cancer Chemotherapeutic Agents In Vitro and In Vivo: Consideration of the Hazard of Drugs as Possible Mutagens and Carcinogens Causing Second Malignancies[1,2]

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Sister chromatid exchanges (SCEs) induced by chemotherapeutic agents currently used for treating various malignancies were studied in vitro and in vivo. Whether the agents are potentially mutagenic/carcinogenic is discussed on the basis of recent clinicopathological observations on secondary malignancy. In the in vitro studies, the induction of SCEs by various anticancer agents, including three anticancer antibiotics, six alkylating agents, and three antimetabolites, was examined and compared with induction by two known carcinogens, 4-nitroquinoline 1-oxide and *N*-ethyl-*N'*-nitronitrosoguanidine. In the in vivo studies, SCE frequencies were examined in the lymphocytes from 74 blood samples drawn from 40 patients with cancer or leukemia at various times after the agents had been administered alone or in combination. The SCE frequencies induced by the anticancer antibiotics were variable because the drugs had different modes of action: generally, the drugs have strong cytotoxicity that must affect the viability of cells. On the other hand, alkylating agents induced significantly high levels of SCE frequencies in vitro; the modes of SCE inducibility were similar to those of the two carcinogens. The fact that cells were viable up to relatively high molar concentrations of the agents and that high SCE levels persisted for a long period suggests that the lesions induced by alkylating agents are long-lived and may therefore be more frequently involved in mutagenesis. This finding may be compatible with the clinical observation that the vast majority of patients with second malignancy are found in the group treated with alkylating agents. Antimetabolites generally showed only a weak SCE induction or none in vivo and in vitro. [Cancer Treat Rep 69:505–514, 1985]

Recently, there has been much concern that cancer chemotherapy may have undesirable consequences in the form of second malignancies. At the beginning of the 1970s, when reports of patients with second malignancies began to appear in the literature, Waldenström (1) cautioned against the trend of cancer chemotherapy. Rosner and Grünwald (2) were probably the first to collect a considerable number of patients in whom acute leukemia developed after treatment of the primary disease. In 1975, Sieber and Adamson (3) made a comprehensive study on the late effects of antineoplastic agents. In the same year the National Cancer Institute held a symposium entitled "Delayed Consequence of Cancer Chemotherapy," which was followed by editorials in several journals. Whether cancer chemotherapeutic agents increase the risk of second malignancy cannot be decided until the incidence in untreated patients has been determined. However, the association between cancer chemotherapy and the development of second malignancy seems to be no longer ambiguous. The accumulation of clinical cases indicates that a causal relation between treatment and leukemogenesis exists (4–10).

In an attempt to understand the basis for this therapy-linked leukemia, the present studies were designed to determine the action of anticancer drugs from the molecular cytogenetic point of view. We report sister chromatid

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exchanges (SCEs) induced by cancer chemotherapeutic agents currently used for treating a variety of malignancies. The studies were designed to elucidate the hazard of drugs as possible mutagens and carcinogens that would be expected from the inherent physiochemical nature of the drugs. In the in vitro experiments SCEs induced by two known carcinogens, 4-nitroquinoline 1-oxide (4-NQO), which has an ultraviolet-like action, and *N*-ethyl-*N'*-nitronitrosoguanidine (ENNG), a monofunctional alkylating agent, were also examined for comparison.

MATERIALS AND METHODS

The experiments were performed in vitro and in vivo. In the former, peripheral lymphocytes from normal subjects were cultured in RPMI-1640 medium supplemented with 15% fetal calf serum and phytohemagglutinin (Wellcome). Doxorubicin (ADM), mitomycin (MMC), zinostatin (NCS), nimustine (ACNU), busulfan (BUS), mitobronitol (DBM), melphalan (MPN), pipobroman (PIPO), procarbazine (PCZ), cytarabine (Ara-C), 5-FU, 6-mercaptopurine riboside (6-MP-R), 4-NQO, and ENNG were dissolved in RPMI-1640, 1% ethanol, or 1% acetone and added to the culture bottles to make a medium containing the chemicals at concentrations of 10^{-3} to 10^{-11} M. Twenty-four hours after the start of the culture, 5-bromo deoxyuridine (BRdU) was added to each culture bottle to give a final concentration of 5 μg/ml.

In the in vivo experiments, blood was taken at various intervals from 40 patients with cancer or leukemia who had been treated with certain chemotherapeutic agents in a single or combination regimen. No patient had received treatment within 2 weeks prior to the current history. The SCE frequencies in cultured lymphocytes from 15 normal individuals (ten male and five female) and from 17 related and unrelated patients (ten male and seven female) with various malignancies before treatment were also investigated. Whole blood was cultured by the same procedure described above.

In both the in vitro and in vivo experiments, all of the cultures were grown in the dark to avoid producing SCEs by photolysis of BRdU-substituted DNA. After two rounds of replication, demecolcine (0.02 μg/ml) was added to each culture 2 hours before harvest. After the cells were treated with a hypotonic solution, they were fixed by several changes of methanol/acetic acid and dropped onto clean and chilled slides that were dried in warm air.

The differential staining of sister chromatids was performed according to a minor modification of the method described by Perry and Wolff (11). In each sample, 40–50 metaphase cells showing differences in chromatid staining were photographed and the SCEs were analyzed. Terminal SCEs were recorded as one exchange and interstitial SCEs were recorded as two. SCEs in the centromeric region were not scored because they were indistinguishable from the twisting of sister chromatids.

RESULTS

The frequency of SCEs in chemical-free, healthy donors used in the in vitro experiment ranged from 6.31 to 8.60. SCE frequencies induced by chemicals and anticancer agents in vitro are shown in table 1 and figure 1.

One of the two carcinogens, ENNG, had relatively low cytotoxicity and an adequate number of mitoses for counting SCEs was available up to 10^{-4} M. Between 10^{-11} and 10^{-4} M, the yield of SCEs was dose-related; the highest value (19.77 ± 4.95) was recorded at 10^{-4} M. Between 10^{-11} and 10^{-5} M, SCEs increased dependent on the dose of 4-NQO; the highest value of SCEs was 24.38 ± 6.17 at 10^{-5} M. Statistically significant increases in SCEs at a given concentration are marked by asterisks and daggers in table 1.

Two of the three anticancer antibiotics, MMC and ADM, were strongly cytotoxic, and the SCEs produced by them could be counted only up to 10^{-7} M, where maximum scores of 30.40 ± 7.17 and 17.79 ± 6.49 were recorded, respectively. SCE frequencies induced by these agents were also dose-related. On the other hand, the third antibiotic, NCS, a protein anticancer antibiotic (molecular weight, approximately 10,700) known to degrade DNA, did not elevate the SCE scores at any concentration examined, although it was most cytotoxic among the chemicals examined. NCS, however, induced numerous exchange type aberrations (12). DBM, an alpha-omega-substituted hexitol that acts as an alkylating agent by forming a diepoxide, yielded the highest mean number of SCEs: 47.49 ± 9.95/cell at 10^{-6} M. Three alkylating agents, MPN, BUS, and ACNU, also showed a dose-related increase in SCEs between 10^{-11} and 10^{-5} M; the maximum SCE yield was almost the same for all three and was similar to those induced by 4-NQO and ENNG. A piperazine derivative, PIPO, on the other hand, produced a slight increase in SCEs at 10^{-6} M. PCZ, which is also a class of alkylating agent, showed the lowest cytotoxicity among all chemicals examined and the SCE frequency produced by it could be counted up to 10^{-3} M, at which a significantly high level was recorded.

Among the three antimetabolites, Ara-C did not increase SCEs at any concentration, whereas 5-FU and 6-MP-R induced SCEs slightly; the increases, although statistically significant at some doses, were not dose-related (table 1).

The SCE frequencies in patients with malignancies before treatment are compared to those of normal individuals (table 2). The SCE frequencies in 15 normal subjects ranged from 6.21 to 8.93/cell, with a mean value of 7.73. The frequencies of SCEs before treatment in related and unrelated patients with various malignancies ranged from 6.70 to 8.73/cell (mean, 7.50 ± 0.69) in males and from 6.13 to 7.96/cell (mean, 7.29 ± 0.64) in females. The sex difference was not significant and the frequencies were in good agreement with those observed in normal

TABLE 1.—SCEs induced by cancer chemotherapeutic agents in vitro*

Drugs	10^{-3} M	10^{-4} M	10^{-5} M	10^{-6} M	10^{-7} M	10^{-8} M	10^{-9} M	10^{-10} M	10^{-11} M	Control
MMC	ND	EI	EI	EI	30.40 ± 7.17†	16.56 ± 3.44†	11.50 ± 2.21†	8.88 ± 2.40‡	7.40 ± 2.87	7.36 ± 2.55
ADM	ND	EI	EI	EI	17.79 ± 6.49†	9.09 ± 3.18‡	6.97 ± 2.89	6.68 ± 2.56	7.29 ± 3.34	7.18 ± 2.68
NCS	ND	ND	EI	EI	EI	6.20 ± 2.55	5.42 ± 3.24	4.90 ± 3.40	4.60 ± 2.42	6.31 ± 2.18
DBM	ND	EI	EI	47.49 ± 9.95†	14.58 ± 5.04†	10.45 ± 4.12‡	8.62 ± 3.36	8.05 ± 3.74	ND	7.74 ± 3.19
PIPO	ND	ND	EI	12.50 ± 4.13†	6.96 ± 1.90	5.56 ± 1.23	6.78 ± 3.07	5.00 ± 1.07	6.05 ± 2.80	6.31 ± 2.18
BUS	EI	EI	22.22 ± 4.35†	12.04 ± 4.89†	1.59 ± 3.49†	9.74 ± 3.83	9.91 ± 3.37‡	7.68 ± 3.29	6.81 ± 2.99	8.60 ± 2.62§
ACNU	EI	EI	20.50 ± 5.27†	7.84 ± 3.01	5.73 ± 2.84	6.66 ± 1.63	4.00 ± 3.69	6.42 ± 3.69	5.07 ± 1.32	6.31 ± 2.18
MPN	EI	EI	24.83 ± 7.29†	9.35 ± 2.54‡	7.08 ± 1.55	6.57 ± 1.44	6.50 ± 1.26	6.28 ± 2.13	6.12 ± 2.03	6.31 ± 2.18
PCZ	16.12 ± 4.75†	7.56 ± 2.24	6.20 ± 3.32	6.67 ± 2.27	6.16 ± 2.48	5.56 ± 2.51	6.04 ± 2.65	6.00 ± 1.85	5.36 ± 2.48	6.31 ± 2.18
5-FU	EI	EI	7.87 ± 2.90	9.63 ± 3.31‡	10.05 ± 4.04‡	9.04 ± 4.16‡	7.87 ± 2.94	8.62 ± 2.61‡	6.63 ± 3.11	7.18 ± 2.68
6-MP-R	ND	9.48 ± 3.40‡	10.23 ± 3.57‡	9.00 ± 3.78	9.48 ± 4.11‡	8.03 ± 2.93	9.11 ± 3.02‡	11.62 ± 4.92†	10.79 ± 4.30‡	7.74 ± 3.19
Ara-C	ND	EI	6.33 ± 2.47	6.57 ± 2.52	5.72 ± 1.92	5.08 ± 1.71	6.03 ± 2.45	5.51 ± 2.45	5.50 ± 3.05	7.18 ± 2.68
4-NQO	EI	EI	24.38 ± 6.17†	3.46 ± 4.66†	11.07 ± 4.38‡	10.68 ± 3.86	9.92 ± 3.05	9.77 ± 3.88	8.81 ± 3.49	7.97 ± 3.42¶
ENNG	EI	19.77 ± 4.95‡	14.11 ± 4.67†	11.30 ± 3.89†	8.97 ± 3.15	8.44 ± 2.52	7.90 ± 1.79	7.61 ± 2.38	7.43 ± 2.65	7.97 ± 3.42¶

* ND = not done; EI = examined but inadequate No. of metaphases observed.
†$P < 0.001$.
‡$P < 0.01$.
§ Control + 1% acetone.
¶ + 1% ethanol.

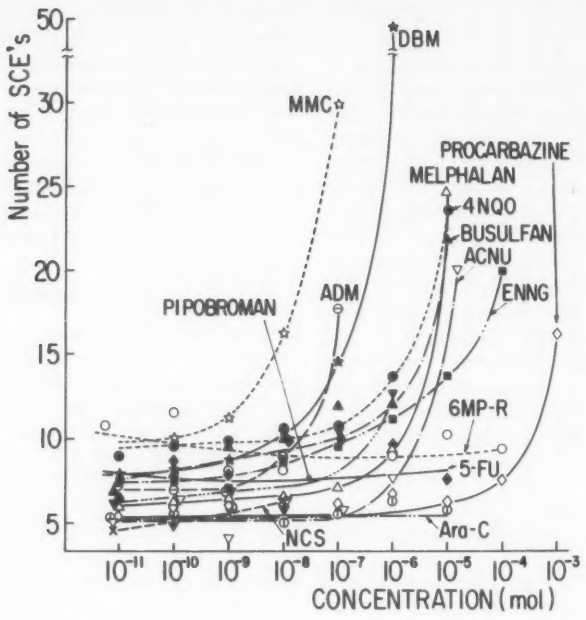

FIGURE 1.—SCE frequencies induced by 3 anticancer antibiotics, 6 alkylating agents, 3 antimetabolites, and 2 carcinogens in vitro.

control subjects. Thus, the frequency of SCEs in normal individuals and in cancer-bearing patients before treatment in our laboratory was always approximately seven to eight per cell and never beyond ten, which we regarded to be a baseline. Increased SCE scores were counted in most patients who received medications for the treatment of primary malignancy. The SCE frequencies in the lymphocytes from patients with cancer or leukemia who had received various chemotherapeutic agents in vivo are summarized in table 3.

Among the treatments, MMC or combined chemotherapy including MMC induced the highest numbers of SCEs. In Patients 1 and 2, SCEs were counted 24 hours after one injection of 4 and 10 mg of MMC, respectively. In Patients 3-7, combined chemotherapy regimens including MMC were used. A high number of SCEs were counted in each patient. Patient 4 showed a dose-related increase in SCEs when repeated samplings were taken during the course of treatment (fig 2, A). Thus, it can be concluded that a major portion of the SCEs obtained by combination chemotherapy is due to MMC, since antimetabolites were unlikely to influence the elevation of SCEs (table 2).

CPM in a single or combined regimen also induced a high frequency of SCEs, but it was not as high as that produced by MMC (table 3). Patients 8 and 9 received a total of 700 and 1950 mg of CPM orally, respectively, and the frequency of SCEs remained near control levels. Patient 10 received a total of 3500 mg of CPM orally and a slight increase in SCE frequency was observed. On the other hand, in Patient 11 a moderate increase in SCEs was observed in a blood sample obtained 24 hours after one injection of 500 mg of CPM. The cumulative effect observed in Patients 10 and 11 is shown in figure 2, D. In Patients 12 and 14, SCEs were also counted repeatedly in the course of treatment; a cumulative effect was not prominent (fig 2, C). Whether a portion of SCEs could be attributed to drugs other than CPM is not known. Probably PCZ and VCR synergistically played some role in producing SCEs, because the SCE yield induced by the oral administration of CPM in small daily doses was not as high as in those patients in whom combination chemotherapy was used.

The effects produced by two anticancer antibiotics, BLM and DNR, and a variety of alkylating agents are given in table 3. BLM, when administered in a small dose, induced a slight increase in the level of SCEs. The other anticancer antibiotic, DNR, in combination with Ara-C, 6-MP, and Pred [DCMP, the most popular and commonly used protocol for acute nonlymphocytic leukemia (ANLL) in Japan] has strong cytotoxicity; this regimen caused a slight increase in SCEs. This unexpectedly low frequency of SCE might be attributable to the cytotoxic effect of DCMP. Patient 19 received an iv injection of 250 mg of

TABLE 2.—SCE frequencies in normal subjects and in related and unrelated patients with malignancies prior to treatment

Patient No.	Sex	No. of cells examined	SCEs		Diagnosis
			Mean ± SD	Range	
			Controls		
1	M	49	6.21 ± 2.72	3-11	
2	M	27	6.93 ± 3.85	1-15	
3	M	30	7.57 ± 3.54	3-11	
4	M	71	7.29 ± 3.05	2-15	
5	M	50	7.74 ± 3.19	3-15	
6	M	43	8.23 ± 3.29	3-17	
7	M	109	7.18 ± 2.68	1-15	
8	M	128	7.36 ± 2.55	2-15	
9	M	21	8.72 ± 3.14	5-15	
10	F	34	7.47 ± 3.31	1-17	
11	F	43	7.07 ± 3.06	1-17	
12	F	39	8.21 ± 3.30	3-17	
13	F	47	8.26 ± 3.56	2-20	
14	F	69	8.93 ± 3.60	3-18	
15	F	45	8.82 ± 3.32	2-16	
			Patients		
1	M	21	7.64 ± 2.38	3-12	Non-Hodgkin's lymphoma
2	M	20	7.77 ± 2.95	3-12	Chronic myelocytic leukemia
3	M	25	7.92 ± 4.32	2-17	Lung cancer
4	M	44	8.36 ± 3.09	5-21	Gastric cancer
5	M	41	7.59 ± 3.29	2-17	Gastric cancer
6	M	37	6.70 ± 3.45	3-19	Gastric cancer
7	M	23	8.73 ± 3.02	3-17	Gastric cancer
8	M	48	7.92 ± 4.71	3-17	Gastric cancer
9	M	42	6.79 ± 3.04	2-14	Gastric cancer
10	M	54	7.38 ± 2.92	3-12	Acute myelocytic leukemia
11	F	20	7.76 ± 2.28	5-13	Acute myelocytic leukemia
12	F	45	7.90 ± 3.42	2-14	Gastric cancer
13	F	46	6.13 ± 2.96	2-17	Lung cancer
14	F	46	7.02 ± 3.21	2-16	Colon cancer
15	F	46	6.43 ± 2.22	3-12	Multiple myeloma
16	F	31	7.55 ± 2.79	2-13	Colon cancer
17	F	24	7.96 ± 3.51	3-15	Breast cancer

ACNU; there was a very high yield of SCEs. In accordance with the in vitro result, DBM yielded a high number of SCEs as a probable consequence of the special chemical structure of the drug. Small doses of the three alkylating agents, MPN, BUS, and PIPO, given orally, induced almost similar, small numbers of SCEs (fig 2, E and F): MPN was the most potent and PIPO was the weakest. PCZ showed weak inducibility of SCEs in vivo as well as in vitro (fig 2, B).

Finally, the effect of the antimetabolites, Pred, and two immunomodulators (NK-471 and Psk) on SCE induction is presented in table 3. In Patients 32 and 36, the SCEs were somewhat elevated, but they remained at control levels in the remaining patients. This was true even in Patient 34, in whom a considerably high dose of the drug was administered. These observations indicate that antimetabolites essentially have no effect on the production of SCEs. The two immunomodulators did not induce SCEs. We have recently found that these two agents and interferon (α, β) inhibit SCEs induced by chemotherapeutic agents in vivo and in vitro; these results will be reported elsewhere.

Figure 3 shows the SCE yield counted repeatedly in two patients who received one injection of 4 mg of MMC (Patient 1), 500 mg of CPM (Patient 11), and 250 mg of ACNU (Patient 19), respectively. In each case a maximum elevation was observed 24 hours after the injection. In the patient receiving MMC, the number of SCEs gradually decreased within 1 week. In the patient receiving high-dose CPM, the effect persisted for about 1 month; this result was similar to that observed after continuous oral administration of the agent in small doses. ACNU had an extremely profound and prolonged effect.

DISCUSSION

A vast number of cases of secondary leukemia (SL) have been reported to occur after chemotherapy with alkylating agents such as MPN, CPM, chlorambucil, and mechlorethamine (2,4-10,14-17). This observation prob-

TABLE 3.—SCE frequencies observed in patients who received various cancer chemotherapeutic agents alone or combined

Patient No.	Chemotherapy: Drug,* daily dose (mg), route	Chemotherapy: Total dose (mg), schedule (days posttreatment)	SCE (mean ± SD)	Diagnosis
1	MMC, 4, iv	4,1 injection (1)	12.21 ± 4.01	Gastric cancer
2	MMC, 10, iv	10, 1 injection (1)	17.44 ± 5.82	Hepatoma
3	MMC, 2, iv FT-207, 600, oral	38, 2/wk (67) 54,200, daily (92)	19.04 ± 7.73	Gastric cancer
4	MMC, 2, iv Ara-C, 20, iv FT-207, 600, oral	4, 2/wk (8) 40, 2/wk (8) 3200, daily (8)	23.80 ± 5.60	Gastric cancer
5	MMC, 2, iv Ara-C, 20, iv FT-207, 600, oral	10, 2/wk (15) 100, 2/wk (15) 4800, daily (8)	25.34 ± 8.94	Gastric cancer
6	MMC, 4, iv Ara-C, 40, iv	24, 2/wk (19) 240, 2/wk (19)	30.67 ± 9.09	Gastric cancer
7	MMC, 4, iv FT-207, 400, iv	24, 2/wk (23) 9200, daily (23)	32.58 ± 6.15	Gastric cancer
8	CPM, 100, oral	700, daily (7)	7.80 ± 3.99	Multiple myeloma
9	CPM, 50, oral	1950, daily (39)	9.12 ± 4.07	Acute myelocytic leukemia
10	CMP, 300, 200, iv	3500, daily (15)	13.00 ± 5.10	Multiple myeloma
11	CPM, 500, iv	500, 1 injection (1)	15.84 ± 4.15	Multiple myeloma
12	CPM, 50, oral 6-MP, 50, oral Pred, 30, oral VCR, 1, iv	750, daily (15) 750, daily (15) 450, daily (15) 1, 1 injection (9)	10.24 ± 2.60	Non-Hodgkin's lymphoma
13	CPM, 50, oral 6-MP, 100, oral Pred, 30–5, oral	24,000, daily (480) 48,000, daily (480) 4800, daily (480)	13.16 ± 4.81	Non-Hodgkin's lymphoma
14	CPM, 50, oral PCZ, 50, oral Pred, 40–30, oral VCR, 1, iv BLM, 15, im	1550, daily (31) 1550, daily (31) 1240, daily (31) 1, 1 injection (31) 60, 1/wk	16.96 ± 3.82	Non-Hodgkin's lymphoma
15	CPM, 50, oral PCZ, 50, oral Pred, 40–30, oral BLM, 15, im	3150, daily (63) 1650, daily (33) 1540, daily (63) 120, 1/wk (63)	24.09 ± 7.41	Non-Hodgkin's lymphoma
16	BLM, 5, iv	30, daily (6)	11.26 ± 4.54	Lung cancer
17	DNR, 40, iv Ara-C, 120, iv 6-MP, 100, oral Pred, 60, oral	160, daily (4) 480, daily (4) 400, daily (4) 240, daily (4)	11.09 ± 4.41	Blastic chronic myelocytic leukemia
18	DNR, 40, iv Ara-C, 120, iv 6-MP, 100, oral Pred, 60, oral	160, daily (4) 480, daily (4) 400, daily (4) 240, daily (4)	11.72 ± 3.50	Chronic myelocytic leukemia in blast crisis
19	ACNU, 250, iv	250, 1 injection (1)	26.50 ± 5.90	Chronic myelocytic leukemia
20	DBM, 150, oral	5100, daily (34)	26.04 ± 6.12	Chronic myelocytic leukemia
21	BUS, 2, oral	654, daily (327)	11.58 ± 4.01	Chronic myelocytic leukemia in chronic phase

continued

TABLE 3.—SCE frequencies observed in patients who received various cancer chemotherapeutic agents alone or combined (*continued*)

Patient No.	Chemotherapy: Drug,* daily dose (mg), route	Chemotherapy: Total dose (mg), schedule (days posttreatment)	SCE (mean ± SD)	Diagnosis
22	BUS, 2, oral	342, daily (171)	11.23 ± 3.12	Polycythemia vera
23	PIPO, 40, oral	280, daily (7)	7.32 ± 2.28	Polycythemia vera
24	PIPO, 40, oral	1360, daily (34)	9.09 ± 2.62	Polycythemia vera
25	PIPO, 40, oral	2000, daily (50)	10.37 ± 1.40	Polycythemia vera
26	MPN, 1–2, oral	44, daily (31)	12.30 ± 4.49	Multiple myeloma
27	PCZ, 50, oral	350, daily (7)	6.90 ± 1.51	Polycythemia vera
28	PCZ, 100, oral	2100, daily (21)	11.55 ± 3.01	Polycythemia vera
29	5-FU, 400, oral	112,000, daily (28)	7.42 ± 3.55	Lung cancer
30	5-FU, 300, oral	3600, daily (12)	7.66 ± 3.15	Gastric cancer
31	5-FU, 400, oral	58,700, daily (179)	8.59 ± 2.81	Gastric cancer
32	5-FU, 250, oral	5500, daily (22)	10.21 ± 4.31	Lung cancer
33	FT-207, 600, oral	36,000, daily (60)	7.97 ± 2.81	Gastric cancer
34	FT-207, 1500, anal	118,500, daily (79)	8.55 ± 2.89	Gastric cancer
35	FT-207, 800, oral	22,400, daily (28)	8.87 ± 2.89	Gastric cancer
36	FT-207, 800, oral	27,200, daily (34)	10.06 ± 4.91	Gastric cancer
37	6-MP, 50, oral Pred, 15, oral	2800, daily (56) 840, daily (56)	7.68 ± 5.59	Malignant lymphoma
38	Pred, 10, oral	770, daily (77)	9.29 ± 3.35	Multiple myeloma
39	NK-421, 30, oral	1890, daily (63)	4.70 ± 1.11	Multiple myeloma
40	Psk, 6000, oral	54,000, daily (9)	6.26 ± 2.36	Gastric cancer

* CPM = cyclophosphamide; 6-MP = 6-mercaptopurine, Pred = prednisolone, VCR = vincristine; BLM = bleomycin; and DNR = daunorubicin.

ably reflects the fact that these agents are the most commonly used for cancer treatment. The primary malignancy itself may be a risk factor, since multiple myeloma and ANLL, for example, have often been observed to occur simultaneously (13). However, the potential relevance of alkylating agents in the development of SL is found in a variety of malignant, as well as in certain nonmalignant, disorders (14). In addition, a positive correlation between initial drug dose and the risk of occurrence of ANLL has been suggested (15).

Alkylating agents are obviously incriminated in Hodgkin's disease. Boiron et al (16) reported that they were used in 96% and PCZ was used in 79% of patients developing second malignant neoplasms (SMNs). Similarly, MPN is so often incriminated that MPN-induced ANLLs are found to be closely associated with M_1 (myelomonocytic) leukemic in the French, American, British classification (17).

The difference in the frequency of SMNs and SL observed between patients with Hodgkin's disease treated with MOPP (mechlorethamine, VCR, PCZ, and prednisone) and those treated with ABVD (ADM, BLM, vinblastine, and dacarbazine) has been documented frequently. Valagussa et al (10) reported that among 764 patients with Hodgkin's disease treated with radiotherapy, chemotherapy, or a combination of the two, ANLL occurred only in the group treated with MOPP or a modified MOPP regimen. They did not observe either SMNs or SL in patients treated with ABVD. Since the difference in the risk of developing ANLL between two treated groups is so clear-cut, MOPP must have some incriminating agents.

In this connection, two reports on SL developing after alkylating agent therapy for polycythemia vera are interesting. In particular, the report of Berk et al (7) on a long-term randomized controlled trial using chlorambucil was rather shocking: the protocol was stopped because of an unexpectedly high actuarial risk of the occurrence of SL that exceeded the highest incidence reported with ^{32}P. In a second report Najman et al (18) observed no cases of SL in 74 patients with polycythemia vera treated with PIPO.

So far, it is not known by what mechanisms cancer chemotherapeutic agents induce second malignancy. Physiochemical (carcinogenic) properties of the agents or their immunosuppressive action (19) may be involved. In view of the clinical observations that have already been

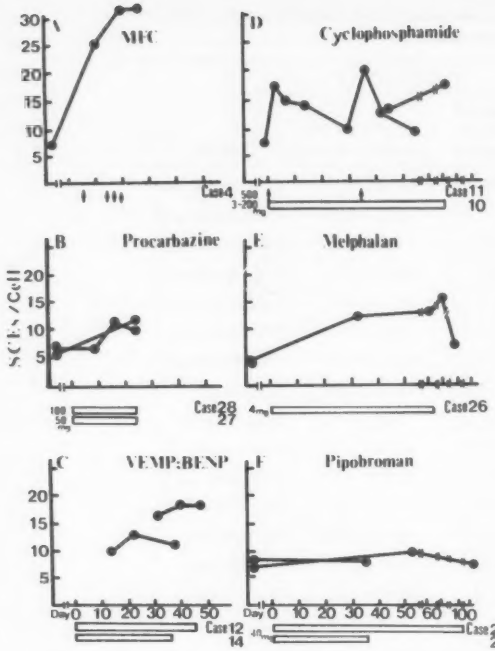

FIGURE 2.—Cumulative effects of 3 combination chemotherapy regimens [MMC + 5-FU + Ara-C (MFC); VCR + CPM + 6-MP + Pred (VEMP); and BLM + CPM + PCZ + Pred (BENP)] and 4 alkylating agents on the production of SCEs in vivo. Patient 4 received 14 mg of MMC, 9200 mg of 5-FU, and 140 mg of Ara-C. Patients 12 and 14 received 5 mg of VCR, 1800 mg of CPM, 1800 mg of 6-MP, and 1080 mg of Pred; they also received 90 mg of BLM, 2250 of CPM, 2250 mg of PCZ, and 1660 mg of Pred.

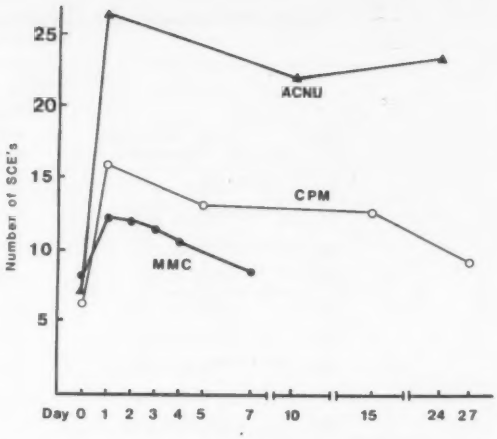

FIGURE 3.—Changes in SCE frequencies after 1 iv treatment with ACNU, CPM, or MMC in vivo.

mentioned, it becomes quite evident that certain chemotherapeutic agents are preferentially involved in leukemogenesis and carcinogenesis, in which the classical error-prone and misrepair concept must have validity. Therefore, the nature of chemotherapeutic agents giving rise to late effects must be discussed from both the cellular and molecular cytogenetic points of view.

A certain chemical that induces a high frequency of SCEs may be mutagenic (20) and therefore carcinogenic (21), but there is no ultimate evidence for this in man. However, few patients with SMNs or SL due to MMC, a most potential SCE inducer, have been reported. The mechanism of action of MMC is similar to classical alkylating agents and seems to involve alkylation and the formation of interstrand cross-link in DNA; however, MMC is somewhat different in that it is strongly cytotoxic. The iv injection of 4 mg of MMC twice a week or 20 mg every 2 weeks results in severe side effects of leukopenia and thrombocytopenia within 1 month. This effect may explain why SMNs have been less frequently observed in the MMC-treated group.

In the present study, the combination of DNR with Ara-C, 6-MP, and Pred increased SCEs slightly in vivo in two patients with chronic myelocytic leukemia blast crisis. These findings agree with those of Lambert et al (22), who reported that a similar anthracycline derivative, ADM, given repeatedly with 5-FU and VCR, produced a very low SCE frequency. In our in vitro experiment, ADM induced a statistically significant high level of SCEs, which was much lower than that induced by MMC at the same molar concentration, suggesting that lesions induced by the former agent were longer-lived than those induced by MMC. Contrary to our findings, Mulsilová et al (23) reported high levels of SCEs lasting as long as 10 weeks after treatment with ADM was stopped.

The carcinogenic potential of the two remaining anticancer antibiotics, BLM and NCS, cannot be sufficiently discussed here. Unlike the findings reported by others (24), BLM slightly increased the SCE frequencies in the patients in our study, whereas NCS did not cause any increase but did produce numerous exchange-type chromosome aberrations (12). Both agents have a similar action in that they can produce double-strand and chromosome breaks. No second malignancies have been reported after BLM treatment.

Chromosome aberration formation and SCEs are seemingly different cellular processes (25). In fact, Gebhardt (26) has shown the disparities between data obtained from chromosome aberration formation and those obtained from SCEs. Of 215 chemicals, 25% showed a poor correspondence and 30.1% showed none. In addition, he classified antimetabolites such as Ara-C, 5-FU, FT-207, and 6-MP in the group that had a significant number of aberrations and few SCEs. It seems probable that Ara-C, for example, causes a marked SCE increase immediately after it is administered (27). After induction chemother-

apy, antimetabolites form a much higher number of micronuclei in the bone marrow of leukemic patients than is observed in untreated patients or in those treated with other than anticancer agents (28). However, in accordance with previous reports (29,30), the numbers of SCEs induced by antimetabolites were within normal limits or only somewhat increased. These findings may correlate with clinicopathological observations that second malignancies, including SL, have been rarely reported after treatment with antimetabolites (31).

The focus of the present studies was the carcinogenicity of alkylating agents and elucidating whether they are the most probable candidates for inducing second malignancies. In the in vitro experiments six alkylating agents were examined. In the case of DBM and PIPO, the cells were viable up to 10^{-6} M, where DBM recorded the highest SCE scores. Because of its limited clinical application there is no information on related second malignancies after treatment with this agent. SCEs induced by PIPO remained slightly higher than the control value. MPN, BUS, and ACNU also showed high levels of SCEs at 10^{-5} M; the levels were comparable to those induced by 4-NQO and ENNG. Because it causes severe and prolonged hematological side effects, ACNU cannot be used continuously or frequently in clinical practice. However, a single administration revealed persistence of high levels of SCEs (fig 3). It seems that a similar nitrosourea compound, lomustine, could cause prolonged and profound increases in SCE frequencies (22,27). This prolonged effect by lomustine may be followed by MPN (22) and CPM (30).

From these observations it becomes obvious that the SCE frequencies are by no means related to the grade of carcinogenicity. SCEs may be considered as a reflection of damages and subsequent repair processes that occurred in the DNA, while not taking into account an extreme view of Brandley et al (32) that they do not represent any mutagenic process. Interstrand cross-linked lesions induced by alkylating agents are supposed to have difficulties in undergoing repair so that lesions are often related to cell death, whereas monofunctional substitution may frequently lead to mutation (33). The present results may be compatible with this concept. If SCEs directly reflect the process of mutagenicity and carcinogenicity, antitumor agents such as MMC and DBM, which induce a higher frequency of SCEs than potential carcinogens (34), would be the most suitable candidates as carcinogens.

The observation of Goth-Goldstein (35) and the postulation of Wolff (25) that lesions leading to SCEs might be concerned with removal and/or repair of the O^6-alkylguanine is interesting; such lesions comprise a minor fraction of SCEs. Sasaki (36) postulated two different ways of SCE formation: the major mechanism is replication bypass repair and the minor mechanism is postreplication repair. The latter is likely to be error-prone. For these reasons chemotherapeutic agents that induce a significant level of SCEs would be carcinogenic. However, as has been repeatedly discussed, the probability of the occurrence of SL or SMNs may largely depend on the viability of the cells affected by the cytotoxicity of the agents (37). It depends on whether lesions caused by a certain anticancer agent are long-lived. Clinically, most incriminated agents such as MPN, BUS, chlorambucil, and CPM, with the exception of ACNU, usually have a mild cytotoxic action that permits their use for a considerably longer period than anticancer antibiotics in general. Therefore, they have more opportunities to be involved in the mutation process. In cases of nitrosourea compounds the effect of a single administration is remarkedly profound and prolonged.

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Cross-Resistance of Vinblastine- and Taxol-Resistant Mutants of Chinese Hamster Ovary Cells to Other Anticancer Drugs[1,2]

Radhey S. Gupta[3,4,*]

Stable mutants resistant to the anticancer drug vinblastine (Vin^R mutants) have been isolated after a single selection step from Chinese hamster ovary cells. Of the two types of Vin^R mutants which are obtained, one class exhibits specific cross-resistance to only some of the microtubule inhibitors. However, the second type of mutants, which is affected in membrane permeability, exhibits cross-resistance to a wide variety of unrelated compounds. Both classes of Vin^R mutants showed codominant expression in cell hybrids formed between resistant and sensitive cells. The cross-resistance patterns of the Vin^R mutants and a single-step mutant resistant to the anticancer drug taxol (Tax^R mutant) toward various anticancer drugs have been determined. The mutants resistant to both of these drugs showed significantly increased resistance toward aclarubicin, dactinomycin, doxorubicin, bisantrene, bruceantin, chromomycin A_3, demecolcine, colchicine, daunorubicin, ellipticine, emetine, ethidium bromide, maytansine, mithramycin, mitoxantrone, nitidine chloride, olivomycin, podophyllotoxin, puromycin, taxol, vinblastine, vincristine, vindesine, teniposide, and etoposide. Interestingly, either one or both of the above mutants exhibited somewhat enhanced sensitivity toward vidarabine, acivicin, bleomycin, cisplatin, cytarabine, α-difluoromethylornithine, 5-FU, tegafur, and tiazofurin. For a number of other anticancer drugs which were examined (chlorambucil, mitolactol, IMPY, hexamethylmelamine, hydroxyurea, diglycoaldehyde, methotrexate, mitoguazone, mitomycin, nocodazole, and 6-thioguanine), the level of resistance of these mutants was found to be unaltered. The information regarding cross-resistance and collateral sensitivity patterns of Vin^R and Tax^R mutants should prove to be very useful in the design of drug combinations which could prove more effective in cancer chemotherapy. [Cancer Treat Rep 69:515–521, 1985]

The development of drug resistance is considered to be one of the major causes for the failure of chemotherapy in the treatment of various kinds of cancers. To prevent development of drug resistance, a common practice in cancer chemotherapy is to employ a combination of different drugs, in the hope that the resistance to the drug combination would not readily occur (1,2). The decision as to which drugs should be used in combination is often based on the criteria that the drugs under consideration act by different mechanisms. In addition, the limited information that is available on the cross-resistance of tumor cells (mostly from experimental animals) to a small number of anticancer drugs is also taken into consideration (3,4). However, our knowledge with regard to the above aspects for many of the drugs is quite limited at present and in recent years a wide variety of new anticancer drugs, whose mechanisms of action are not well-understood, have been introduced for cancer chemotherapy (5–7). In view of the above considerations, to use the available anticancer drugs most effectively, it is exceedingly important to identify the drugs that cells can develop resistance to simultaneously, and the drugs to which cells do not become resistant at the same time.

One approach that has proved to be very useful in this regard involves cell culture model systems in which mutants of human and other mammalian cells are initially selected and examined to determine their cross-resistance to other drugs (8–11). Using this approach, we have

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recently shown that mutants of Chinese hamster ovary (CHO) cells that have been selected for resistance to the anticancer drugs teniposide and etoposide exhibited significant cross-resistance to a wide variety of unrelated drugs (12). The present report describes the selection and partial characterization of CHO cell mutants resistant to the vinca alkaloid vinblastine, which is widely used in the treatment of many different types of cancer (2,5–7,13). The cross-resistance pattern of mutants resistant to vinblastine and another anticancer drug, taxol (14,15), to a wide variety of other anticancer drugs is reported. Some implications of these studies regarding the use of different anticancer drugs in combination chemotherapy are discussed.

MATERIALS AND METHODS

Cell Culture and Cell Lines

The parental CHO cell line, which has been used for mutant selection, is auxotrophic for proline and is usually referred to as wild type (WT) in our work (12,15). The selection of Vin^R mutants is described in the text. The Tax^R-2 mutant was obtained from WT cells after a single selection step in the presence of taxol, as described earlier (15). Except where indicated, the various cell lines were routinely grown at 37°C in alpha minimum essential medium supplemented with 5% fetal calf serum (FCS).

Chemicals

Aclarubicin, acivicin, bisantrene, mitolactol, IMPY, diglycoaldehyde, mitoxantrone, nafoxidine, nitidine chloride, and tiazofurin were provided by Dr. V. L. Narayanan, National Cancer Institute, Bethesda, MD. α-Difluoromethylornithine (α-DFMO) was provided by Dr. P. Bey, Centre de Recherche Merrell International, Strasbourg, France. Vincristine and vindesine were supplied by Dr. G. A. Poore (16), Eli Lilly and Company, Indianapolis, IN. The sources of other anticancer drugs and inhibitors have been described earlier (12,17).

Degree of Resistance of Mutant Cells Toward Various Drugs

The degree of resistance of mutants toward various drugs was determined by seeding about 100 and 200 cells in duplicate into the wells of 24-well tissue culture dishes containing various dilutions of the drugs (including controls which contained no drug) in the growth medium, as described earlier (12,17). The dishes were incubated for 6–7 days at 37°C after which they were stained with 0.5% methylene blue in 50% methanol and the number of colonies was scored. From the averages of the numbers of colonies observed at different concentrations of the drugs, relative plating efficiencies were determined as the ratios of the number of colonies at a given drug concentration to that obtained in the absence of the drug. The D10 value of a drug toward a cell line refers to the dose of the drug which reduces relative plating efficiency of a cell line to 10% as compared to the control cells in the absence of any drug.

Other Procedures

The cell hybrids between resistant and sensitive cells were constructed by a procedure described earlier (18). Cellular uptake of ^{3}H-daunorubicin in sensitive and resistant cell lines was studied, as has been described recently (12,15).

RESULTS

The mutants resistant to vinblastine were obtained by plating CHO cells (1×10^6/100-mm-diameter dish) in medium containing 20 ng/ml of the drug, which kills the sensitive cells (fig 1). The resistant colonies were obtained in these experiments at a frequency of about 1×10^{-6}. The dose-response curves toward vinblastine of four of the resistant clones (Vin^R-1 to Vin^R-4) are shown in figure 1. As can be seen, based on their D10 values for vinblastine, these mutant cell lines are between twofold and fivefold more resistant to vinblastine as compared to the parental WT cells. The drug-resistant phenotype of all of these mutants is maintained after prolonged growth in nonselective medium.

Cross-Resistance of the Vin^R Mutants to Other Microtubule Inhibitors

Since cross-resistance studies with mutants can often provide valuable information regarding the nature of genetic lesions in such cells, cross-resistance of the Vin^R mutants toward a number of other microtubule inhibitors was examined. The results of these studies for two representative mutants, Vin^R-1 and Vin^R-4, are presented in table 1. Other Vin^R mutants examined were found to be similar to one of these mutants. As can be seen, the two Vin^R mutants showed nearly proportional increases in their degree of resistance toward the two vinblastine analogs, vincristine and vindesine. However, for the other microtubule inhibitors which were examined, the two Vin^R mutants showed marked and interesting differences in their sensitivity. For example, for colchicine, demecolcine, and maytansine, the Vin^R-1 line was more resistant in comparison to the Vin^R-4 line, whereas for steganacine and podophyllotoxin the level of resistance of Vin^R-4 was higher in comparison to Vin^R-1. The difference between the two types of Vin^R mutants was most clearly seen in the case of taxol, to which the Vin^R-4 cells showed normal sensitivity, whereas the Vin^R-1 mutant was highly resistant. The two Vin^R mutants also showed

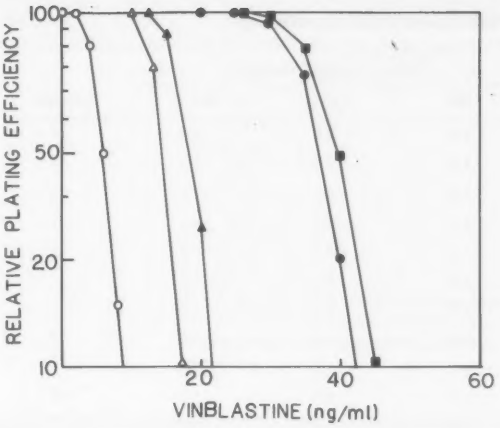

FIGURE 1.—Dose-response curves of WT and some representative vinblastine-resistant lines toward vinblastine. O——O = WT; ●——● = Vin^R-1; ■——■ = Vin^R-2; Δ——Δ = Vin^R-3; and ▲——▲ = Vin^R-4.

slightly increased resistance to griseofulvin, but their sensitivity toward nocodazole was identical to that observed for the parental WT cells (table 1).

Cellular Uptake of ^{3}H-Daunorubicin in Vin^R Mutants

To investigate if the lesion in any of these Vin^R mutants was nonspecifically affecting the membrane permeability of various drugs, cross-resistance of these mutants toward a number of compounds which do not inhibit microtubule functions (eg, puromycin, daunorubicin) was determined. Many of the mutants affected in membrane permeability are known to exhibit cross-resistance to these compounds (8–10,15,19,20). The results of cross-resistance studies with these compounds are shown in table 2. As can be seen, the Vin^R-1 mutant was highly resistant to both of these compounds, whereas sensitivity of the Vin^R-4 cell line to these drugs was similar to the WT cells (results not shown). These studies indicated that the Vin^R-1 mutant was presumably affected in the cellular uptake of various compounds. This possibility was directly tested by examining the cellular uptake of ^{3}H-daunorubicin by various cell lines. The results of these studies are presented in table 3. In the Vin^R-4 mutant, uptake of ^{3}H-daunorubicin was comparable to that in the WT cells, whereas in the Vin^R-1 cell line the cellular uptake of ^{3}H-daunorubicin was greatly reduced (≅ 30% level) at all three concentrations of the labeled drug. Similar results have been obtained in two different experiments with these cell lines. These results strongly indicate that the lesion in the Vin^R-1 mutant is affecting the membrane permeability of various drugs.

Behavior of Vin^R Mutants in Cell Hybrids

Recent studies have shown that while some of the recessive mutants can be readily selected in cell lines such as CHO, which may be functionally hemizygous at the affected loci, the mutants which behave codominantly in cell hybrids are, in principle, readily selectable in all types of cells, including diploid human cells (21,22). Therefore, it was of interest to find out whether the drug-resistant phenotypes of these mutants behaved recessively or dominantly in a $Vin^R \times Vin^S$ situation. This was examined by constructing somatic cell hybrids between the two Vin^R mutants and a vinblastine-sensitive cell line, EOT-3, which contain a number of genetic markers which are useful for selection of hybrids (15,18). Subsequently, the degree of resistance of various hybrid cell lines toward vinblastine was determined. Results of these

TABLE 1.—Cross-resistance pattern of Vin^R mutants toward other microtubule inhibitors

Microtubule inhibitors	D10 for WT cells (μg/ml)*	Relative degree of resistance of the cell lines†		
		WT	Vin^R-1	Vin^R-4
Vincristine	0.020	1	4.2	2.6
Vindesine	0.026	1	4.5	2.7
Colchicine	0.10	1	4.4	1.3
Demecolcine	0.02	1	2.3	1.4
Steganacine	0.13	1	1.3	2.0
Podophyllotoxin	0.008	1	1.7	2.0
Maytansine	0.0008	1	4.5	1.7
Taxol	0.1	1	5.0	1.0
Griseofulvin	4.5	1	1.3	1.2
Nocodazole	0.008	1	1.0	1.0

* D10 value represents the concentration of the drug which reduces plating efficiency of a cell line to 10% of that observed in the absence of any drug. D10 values of various drugs for WT and mutant cell lines were obtained from experiments similar to those described in fig 1.

†Assuming the D10 values of various drugs toward WT cells as 1, relative degrees of resistance of mutant cell lines were obtained as the ratios of the D10 values of the mutant cell lines as compared to the WT cell line. Similar results have been obtained for these drugs in at least 2 separate experiments.

TABLE 2.—Cross-resistance of Vin^R and Tax^R mutants toward other anticancer drugs*

Anticancer drug	NSC No.	D10 value for WT cells (μg/ml)†	Relative degree of resistance†			Group‡
			WT	Vin^R-1	Tax^R-2	
Aclarubicin	208734	0.002	1.0	2.0	4.5	I
Dactinomycin	3053	0.003	1.0	2.5	5.5	I
Doxorubicin	123127	0.05	1.0	4.0	7.0	I
Bisantrene	337766	0.001	1.0	4.0	8.0	I
Bruceantin	165563	0.016	1.0	3.5	6.0	I
Chromomycin A_3	58514	0.06	1.0	5.0	> 8.0	I
Daunorubicin	82151	0.02	1.0	3.0	7.5	I
Ellipticine	71795	0.07	1.0	1.3	1.5	I
Emetine	33669	0.02	1.0	2.0	5.0	I
Ethidium bromide	84423	1.2	1.0	2.5	> 4.0	I
Mithramycin	24559	0.8	1.0	5.0	10.0	I
Mitoxantrone	301739	0.15	1.0	5.0	10.0	I
Nitidine chloride	146397	2.0	1.0	10.0	5.0	I
Olivomycin	76411	10.0	1.0	> 3.0	> 3.0	I
Puromycin	—	2.0	1.0	5.0	> 6.0	I
Teniposide	122819	0.03	1.0	5.0	9.0	I
Etoposide	141540	0.3	1.0	4.5	> 7.0	I
Chlorambucil	3088	50.0	1.0	1.0	1.0	II
Mitolactol§	104800	2.0	1.0	1.0	1.0	II
Hexamethylmelamine	13875	150.0	1.0	1.0	1.0	II
Hydroxyurea	32065	8.0	1.0	1.0	1.0	II
IMPY§	51143	15.0	1.0	1.0	1.0	II
Diglycoaldehyde§	118994	60.0	1.0	1.1	1.0	II
Methotrexate§	740	0.005	1.0	1.0	1.0	II
Mitoguazone	32946	0.03	1.0	1.2	1.0	II
Mitomycin	26980	0.06	1.0	1.0	1.2	II
Nafoxidine	70735	3.0	1.0	1.1	1.0	II
6-Thioguanine§	752	0.05	1.0	1.0	1.0	II
Vidarabine§	404241	8.0	1.0	1.0	0.5	III
Acivicin§	163501	0.07	1.0	0.6	0.5	III
Bleomycin	125066	5.0	1.0	0.4	0.5	III
Cisplatin	119875	1.0	1.0	0.5	0.6	III
Cytarabine§	63878	0.1	1.0	0.6	0.4	III
α-DFMO§	—	5.0	1.0	1.0	0.4	III
5-FU§	19893	0.15	1.0	2.0	0.5	III
Tegafur§	148958	4.0	1.0	2.0	0.5	III
Tiazofurin§	286193	2.0	1.0	0.6	0.5	III

* Cross-resistance data for these mutants for various antimitotic drugs are given in table 1 and in ref 15.

†Described in table 1.

‡Division of these anticancer drugs into 3 groups is described in the text. Group I refers to those drugs to which the Vin^R and Tax^R mutants exhibit significant cross-resistance; Group II refers to drugs for which sensitivity of mutant cells is not altered; and Group III contains anticancer drugs for which either one or both of the above mutants show enhanced sensitivity in comparison to the parental WT cells.

§Sensitivity was determined in medium containing 5% dialyzed FCS instead of FCS.

TABLE 3.—Uptake of ^{3}H-Daunorubicin by the WT and mutant cell lines

Cell line	Concentration of ^{3}H-† daunorubicin ($\times 10^{-7}$ M)	Uptake of radioactivity/10^6 cells*	Uptake relative to WT cells†
WT	1	1774 ± 237‡	100
Vin^R-1	1	557 ± 91	31.4
Vin^R-4	1	1748 ± 142	98.5
WT	2	3890 ± 478	100
Vin^R-1	2	1127 ± 107	29.0
Vin^R-4	2	4008 ± 452	103.0
WT	4	11,391 ± 1007	100
Vin^R-1	4	3740 ± 326	32.8
Vin^R-4	4	11,520 ± 1422	101.1

* Experiment was carried out as described in earlier studies (12,15).

†Assuming average amount of ^{3}H-daunorubicin taken up by WT cell as 100% (at any given concentration of drug in medium), relative uptake in mutant cell lines was calculated.

‡Mean ± SD.

studies for two representative hybrid clones from each of the two crosses (Vin^R-1 × EOT-3 and Vin^R-4 × EOT-3) are shown in figure 2. The hybrid cell lines obtained after fusing Vin^R-1 or Vin^R-4 cells with the EOT-3 line showed levels of resistance toward vinblastine which were intermediate between that of the EOT-3 and the Vin^R mutant cells. These results provide evidence that the drug-resistant phenotypes of both types of Vin^R mutants behave in a codominant manner in cell hybrids.

Cross-Resistance of Mutants Resistant to Vinblastine and Taxol to Other Anticancer Drugs

We next investigated the cross-resistance pattern of the Vin^R mutants toward various other anticancer drugs. In these studies, we also examined cross-resistance of a mutant resistant to another antimitotic, anticancer drug, taxol (Tax^R-2), which has been selected after a single-step selection in earlier studies (15). As reported earlier, like Vin^R-1, the Tax^R-2 mutant is also affected in the membrane permeability of various unrelated drugs and shows a codominant behavior in cell hybrids (15).

The results of cross-resistance studies with the Vin^R-1 and the Tax^R-2 mutants for various anticancer drugs are shown in table 2. The Vin^R-4 mutant, as indicated earlier, exhibited no cross-resistance to unrelated compounds (results not shown). However, both Vin^R-1 and Tax^R-2 mutants exhibited significantly increased resistance toward a number of different anticancer drugs including aclarubicin, dactinomycin, doxorubicin, bisantrene, bruceantin, chromomycin A_3, daunorubicin, emetine, ethidium bromide, mithramycin, mitoxantrone, nitidine chloride, olivomycin, puromycin, teniposide, and etoposide. Both of these mutants also showed a low degree of cross-resistance toward ellipticine in repeated experiments. The level of resistance of the Tax^R-2 mutant to most of these drugs was about twofold higher compared to the Vin^R-1 mutant. The only exception was nitidine chloride, to which the Vin^R-1 line was more resistant in comparison to the Tax^R-2 mutant. In contrast to the above drugs, both of these mutants were found to have become somewhat more sensitive to acivicin, bleomycin, cisplatin, cytarabine, and tiazofurin, as compared to the parental WT cells. These two mutants, however, showed some differences in their pattern of sensitivity toward 5-FU and the related drug tegafur, to which the Vin^R-1 mutant showed slight cross-resistance, while the Tax^R-2 line was found to be more sensitive. The Tax^R-2 mutant also showed enhanced sensitivity toward vidarabine and α-DFMO. For the remaining anticancer drugs which were examined, namely mitolactol, chlorambucil, hexamethylmelamine, hydroxyurea, IMPY, diglycoaldehyde, methotrexate, mitoguazone, mitomycin, nafoxidine, and 6-thioguanine, the level of resistance of the mutant cells was similar to that of the WT cells.

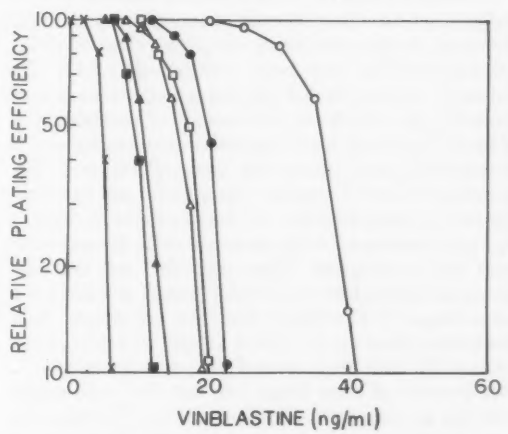

FIGURE 2.—Survival curves of the parental and the Vin^R × Vin^S hybrid cell lines in presence of different concentrations of vinblastine. x——x = EOT-3 (Vin^S) line; O——O = Vin^R – 1; ●——● = Vin^R – 4; △——△ and □——□ = 2 representative hybrid clones from the Vin^R – 1 × EOT-3 cross; and ▲——▲ and ■——■ = 2 representative hybrid clones from the Vin^R – 4 × EOT-3 cross.

DISCUSSION

This paper examines the cross-resistance pattern toward other anticancer drugs of single-step mutants of CHO cells that have been selected for resistance to the anticancer drugs vinblastine and taxol. Our studies show that one class of mutants resistant to both of these drugs, which shows greatly reduced cellular uptake of ^{3}H-daunorubicin, exhibits very similar patterns of cross-resistance to a variety of other anticancer drugs. The anticancer drugs to which these mutants exhibit increased resistance include several antimitotic inhibitors (vinblastine, vincristine, vindesine, taxol, podophyllotoxin, maytansine, colchicine, and demecolcine), a few protein synthesis inhibitors (puromycin, emetine, and bruceantin), and a large number of other compounds (aclarubicin, dactinomycin, doxorubicin, bisantrene, chromomycin A_3, daunorubicin, ethidium bromide, mithramycin, mitoxantrone, nitidine chloride, olivomycin, teniposide, and etoposide), which either intercalate or interact directly with DNA (2). We have earlier observed that CHO cell mutants selected for resistance to teniposide and etoposide (Vpm^R mutants) also show increased cross-resistance to the above anticancer drugs, although the level of cross-resistance of these mutants was lower in comparison to the Vin^R and Tax^R mutants (12; unpublished results). The simultaneous development of resistance to all of the above drugs in single steps in many different types of mutants provides strong suggestive evidence that the cellular uptake of the above compounds occurs by a common mechanism and that these mutants are affected in the transport mechanism.

In earlier studies, cross-resistances of mutants of CHO cells, which have been selected in multiple steps for high levels of resistance to either colchicine, vinca alkaloids, or daunorubicin, have been investigated (8,9,19). The mutants obtained in multiple steps, which were also affected in the membrane permeability of unrelated compounds, exhibited high degrees of cross-resistance to compounds such as puromycin, daunorubicin, taxol, vinblastine, colchicine, emetine, doxorubicin, and etoposide. However, cross-resistance of these mutants to many of the other anticancer drugs examined in the present study was not investigated. Since the Tax^R and the Vin^R mutants studied here were both obtained in single-selection steps, it is evident that the pleiotropic drug-resistance phenotype is not a unique characteristic of cells which have been repeatedly exposed and selected in the presence of these drugs, but that this could readily develop as a result of a single mutation. Furthermore, our observation that the drug-resistant phenotype of both Vin^R and Tax^R mutants is expressed codominantly in cell hybrids formed between resistant and sensitive cells suggests that such mutants may also readily develop in human diploid cells under in vivo situations.

The results of cross-resistance studies presented here also provide valuable information regarding drug combinations to which cellular resistance should not develop readily and which may prove to be more useful in cancer chemotherapy. Based on the cross-resistance patterns of the Vin^R and Tax^R mutants, the various anticancer drugs examined in the present study could be arbitrarily classified into three groups (table 2).

Group I consists of those anticancer drugs to which cellular resistance could develop simultaneously as in the case of Vin^R-1 and Tax^R-2 mutants. This group includes all of the anticancer drugs which intercalate into DNA (aclarubicin, dactinomycin, doxorubicin, bisantrene, chromomycin A_3, daunorubicin, ellipticine, ethidium bromide, mithramycin, mitoxantrone, nitidine chloride, and olivomycin), teniposide, etoposide, a number of antimitotic inhibitors (vinblastine, vincristine, vindesine, taxol, podophyllotoxin, maytansine, colchicine, and demecolcine; table 1), and several protein synthesis inhibitors (puromycin, emetine, and bruceantin). It is obvious that the use of more than one drug from this group in a drug combination would provide no advantage as far as development of drug resistance is concerned.

Group II consists of those drugs to which the mutants exhibiting pleiotropic drug-resistance phenotype do not show any significant degree of cross-resistance. This group includes various drugs which either alkylate or form other adducts with DNA (chlorambucil, mitolactol, hexamethylmelamine, IMPY, mitomycin, and nafoxidine) and a number of antimetabolites and nucleoside analogs (hydroxyurea, diglycoaldehyde, methotrexate, mitoguazone, and 6-thioguanine). The lack of cross-resistance of the Vin^R and Tax^R mutants to these drugs provides strong evidence that the mechanisms by which cellular resistance to these drugs develop is independent and different from those involved in resistance to the Group I drugs. In view of this, the use of one (or perhaps more) drug from this group in combination with a drug from Group I should prove advantageous in prevention of drug resistance.

Group III consists of a number of anticancer drugs and nucleoside analogs for which sensitivity of the mutants exhibiting pleiotropic drug-resistant phenotype is enhanced in comparison to the normal cells. For a number of drugs in this group (acivicin, bleomycin, cisplatin, cytarabine, and tiazofurin) both the Vin^R and the Tax^R mutants show increased sensitivity, whereas for several additional drugs (vidarabine, 5-FU, tegafur, and α-DFMO) enhanced sensitivity is observed only for the Tax^R-2 mutant. In earlier studies, the increased sensitivity toward bleomycin and cisplatin was also observed with the mutants resistant to teniposide and etoposide (12), indicating that this may be a general characteristic of all pleiotropic drug-resistant mutants. This suggests that the same mutation which confers pleiotropic drug resistance may also cause concomitant increased sensitivity to a number of Group III drugs. In view of this observa-

tion, the use of one of the Group III drugs, particularly those to which all the mutants show enhanced sensitivity (bleomycin and cisplatin), in combination with a Group I drug should prove to be very useful in preventing development of pleiotropic drug resistance.

The above considerations suggest that an ideal drug combination to which cellular resistance is not expected to develop readily would perhaps consist of one anticancer drug from each of the three groups (I-III) (table 2). Since there are a number of drugs in each group, a large number of drug combinations suitable for different types of cancers can be developed.

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Cure of Mice Bearing P388 Leukemia by Vincristine in Combination With a Calcium Channel Blocker[1,2]

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Mice inoculated with P388 leukemia were treated initially with vincristine (VCR) at a dose of either 1.5 or 2.0 mg/kg. After 1 week, VCR alone (0.1 mg/kg) was given daily for ten treatments, but no mice were cured with this regimen. However, when the calcium channel blocker verapamil (75 mg/kg) was administered with VCR at 1.5 or 2.0 mg/kg, lifespans were prolonged and one of ten or six of ten mice were cured, respectively. As reported previously, verapamil renders less sensitive or resistant tumor cells susceptible to VCR by inhibiting the VCR efflux mechanism, especially in resistant cells. Therefore, verapamil in combination with VCR apparently can result in total cell kill under the conditions used. [Cancer Treat Rep 69:523–525, 1985]

The heterogeneous nature of cancer is widely recognized in different patients and animals. Different tumor cells within a population usually show inherently different sensitivities to antitumor agents (1–4). The heterogeneous nature of tumor cells causes crucial problems in current cancer chemotherapy, since the proliferation of cells inherently resistant to drugs administered during treatment can lead directly to failure of that treatment.

We previously reported that verapamil, a calcium channel blocker, enhanced the cytotoxicity of vincristine (VCR), vinblastine, and doxorubicin (ADM) in P388 cells and their VCR- and ADM-resistant variants (5,6). Similar results have also been reported in Ehrlich ascites carcinoma by other investigators (7). Subsequent studies revealed that calmodulin inhibitors and other calcium influx blockers also effectively enhanced VCR and ADM cytotoxicity in P388/VCR, P388/ADM, and various human hematopoietic tumor cells (6,8–10). We found that calcium influx blockers and calmodulin inhibitors effectively inhibited the VCR- and ADM-efflux function of tumor cells, especially in drug-resistant tumor cells where the drug efflux mechanism had been enhanced. Verapamil rendered tumor cells less sensitive to VCR more susceptible to the drug and therefore circumvented inherent resistance in vitro (10,11). To explore the possibility of circumventing the heterogeneous nature of tumor cells with respect to drug sensitivity in vivo, we have examined the effect of verapamil in combination with VCR on P388 leukemia-bearing mice.

MATERIALS AND METHODS

P388 leukemia cells were obtained from DBA mice (5,8). Drug-treatment experiments were carried out as described previously (5,8). Briefly, tumor cells (2×10^4) were cultured at 37°C for 5 hours in Falcon No. 2054 culture tubes containing 2 ml of growth medium (RPMI-1640 supplemented with 10% fetal bovine serum). Then cells were treated with graded drug concentrations of VCR (0.1–100 nM), reincubated for 72 hours in the presence of drugs, and counted with a Model ZBI Coulter Counter (8). IC50 (drug concentration required for 50% inhibition of cell growth) was determined (5,8).

In animal experiments, $CD2F_1$ mice were inoculated ip with 10^6 P388 leukemia cells. On the day after tumor inoculation, VCR was administered ip at 1.5 or 2.0 mg/kg. On Day 8 after tumor inoculation the mice were divided into two groups of ten mice each. Mice were treated ip with either VCR alone (100 μg/kg) or with VCR plus verapamil (75 mg/kg). This therapy was continued daily for 10 days. Antitumor activity was evalu-

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TABLE 1.—Combined effect of VCR and verapamil on P388 leukemia-bearing mice*

Drug and dosage (mg/kg)		Mean survival time (days) ± SD of dead mice	% ILS	No. of cured mice (n = 10)
1st therapy	2nd therapy			
Control	—	10.4 ± 0.5	0	0
VCR (1.5)	—	17.6 ± 1.1	69	0
VCR (2.0)	—	18.5 ± 1.5	78	0
VCR (1.5)	VCR (0.1)	22.3 ± 1.5†	115	0
VCR (2.0)	VCR (0.1)	25.5 ± 2.9†	145	0‡
VCR (1.5)	VCR (0.1) + verapamil (75)	36.8 ± 7.4†	253	1
VCR (2.0)	VCR (0.1) + verapamil (75)	30.3 ± 1.9†	192	6‡

*Each group of 10 $CD2F_1$ mice was given ip implants of 10^6 P388 leukemia cells. In the 1st therapy, VCR was administered ip on Day 1 at 1.5 or 2.0 mg/kg. On Day 8, 2nd therapy was conducted with VCR alone or VCR + verapamil. Doses of VCR and verapamil were 100 μg/kg and 75 mg/kg, respectively, and drugs were given ip every day for 10 days.

†Significant ($P < 0.05$) by t test as compared with each value.

‡Significant ($P < 0.05$) by U test as compared with each value.

ated by the mean survival time of each group of mice (5,8).

RESULTS

P388 leukemia cells are heterogeneous with respect to their VCR responses. We isolated 50 clones from P388 leukemia cells and examined their VCR sensitivity by determining the IC50 value for each clone. The IC50 range and number of clones (% of total) within each range were: 0.5–1.0 nM VCR (three clones, 6%); 1.0–1.5 nM (four clones, 8%); 1.5–2.0 nM (19 clones, 38%); 2.0–2.5 nM (18 clones, 36%); 2.5–3.0 nM (three clones, 6%); 3.0–3.5 nM (two clones, 4%); and 3.5–4.0 nM (one clone, 2%).

One million heterogeneous P388 leukemia cells were inoculated into $CD2F_1$ mice. Control mice survived for 10.4 days (table 1). Mice treated with VCR alone survived for longer periods. When initial therapy of 1.5 mg/kg of VCR was followed by secondary VCR therapy, an increase in lifespan (ILS) of 115% was obtained. When the first therapy was 2.0 mg/kg, an ILS of 145% was obtained. No mice were cured with these treatment regimens. However, when the initial therapies of 1.5 and 2.0 mg/kg of VCR were followed by VCR and verapamil (75 mg/kg) in the secondary therapy, ILSs of 253% and 192% were obtained, respectively. These values were significantly higher than those obtained for the groups treated with VCR alone in the secondary phase. Of note was the incidence of cure in these treatment groups (survivors after 6 months were regarded as cured mice). One of ten mice or six of ten mice became tumor free in these groups of mice treated initially with VCR at 1.5 and 2 mg/kg, respectively, followed by secondary therapy with VCR and verapamil combined.

The tumor cells, which regrew in mice treated with VCR alone, were recovered, and the VCR sensitivity of these recurrent cells examined (table 2). IC50 values of VCR against the recurrent cells were 4.3, 9.6, and 16.3 nM. Indices of resistance of the recurrent cells were 2.3, 5.1, and 8.6. Since the IC50 of VCR against drug-sensitive P388 cells was 1.9 nM, these results clearly indicate that the recurrent cells were resistant to VCR after VCR therapy alone.

DISCUSSION

According to Schabel et al (3), VCR therapy at 1.5 mg/kg on Day 1 after the inoculation of 10^6 cells reduces the surviving P388 cells to approximately 4×10^2. The surviving cells, which may be partially resistant to VCR, cannot be killed by secondary therapy with VCR alone, and the mice were finally killed by P388 leukemia resistant to VCR as described above. The secondary therapy using VCR and verapamil killed these partially VCR-resistant cells. In some mice, combination therapy with VCR and verapamil resulted in a significantly high cure rate. Other calcium channel blockers such as nicardine (8) also resulted in partial cures.

Human hematopoietic cells have been classified into several different subtypes by their surface markers and membrane antigens (12–14), and it has been shown that the response to therapeutic agents is different among these subtypes (12–14). The heterogeneous nature of human tumor cells in their response to chemotherapeutic agents is a crucial problem in current cancer chemotherapy (1–4). In most cases, although a complete remission can be achieved, it is very difficult to

TABLE 2.—VCR sensitivity of regrown P388 cells after 2nd therapy with VCR*

Cells	IC50 (nM) of VCR (mean ± SD)	Index of resistance
P388 parent	1.9 ± 0.14	1.0
P388 cells after VCR treatment		
Line 1	4.3 ± 0.22†	2.3
Line 2	9.6 ± 0.34†	5.1
Line 3	16.3 ± 0.54†	8.6

*Mice inoculated with 10^6 P388 cells were treated with VCR at 2 mg/kg in the 1st therapy and at 0.1 mg/kg in the 2nd therapy as described in table 1. Tumor cells were recovered on Days 20–25 from the peritoneal cavity of 1 mouse whose tumor had regrown. Cultured cell lines were established in vitro from the tumor cells of 1 mouse, and VCR sensitivity of 3 successfully established cell lines was determined.

†Significant ($P < 0.05$) by t test as compared with the value of parent cells.

attain a "cure" with regimens employing only chemotherapeutic agents. In the present study, a higher "cure" rate could be obtained by VCR in combination with verapamil. Immunologic host response to transplanted P388 cells could play some role in these cures. Also, verapamil itself, because of its lipophilic side chains, might have some immunostimulatory activity. The significance of immunologic host response in these cures is not known at present; however, the contribution might be small because P388 leukemic cells are known to be highly syngeneic (tumorigenic) with the host utilized (15). This approach indicates a potential for circumventing VCR sensitivity-heterogeneity of hematopoietic tumor cells in a single host.

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Vitamin K_3 Inhibition of Malignant Murine Cell Growth and Human Tumor Colony Formation[1]

Rowan T. Chlebowski,* Marian Dietrich, Steven Akman, and Jerome B. Block[2]

To assess the antineoplastic potential of vitamin K compounds, the effects of vitamin K_3 (menadione), vitamin K_1 (phylloquinone), and warfarin on L1210 murine leukemia cell growth were studied in a flask culture system. When the cytotoxic potential of vitamin K_3 was recognized, the effects of vitamin K_3 on human tumor colony formation were studied in 34 tumor explants using a soft agar (clonogenic) assay system. Complete inhibition of L1210 growth in flask culture was achieved at concentrations of 200 μg/ml of warfarin, 75 μg/ml of vitamin K_1, and 4 μg/ml of vitamin K_3. Combined use of vitamin K and warfarin enhanced cytotoxicity because a concentration of 1 μg/ml of vitamin K_3 together with 70 μg/ml of warfarin resulted in nearly complete inhibition of L1210 growth. Comparable inhibition of growth was seen against malignant murine cell lines in the soft agar assay system, where > 70% decrease in colony formation was seen with vitamin K_3 at concentrations of 6.4 μg/ml for L1210 leukemia and 1 μg/ml for HII4E hepatoma lines. Vitamin K_3 was also cytotoxic in the same dosage range when tested in vitro against the 34 human tumor explants in the soft agar assay system. Tumor types evaluated included adenocarcinoma of the breast (16 patients), ovary (five), colon (two), stomach (two), kidney (two), and unknown primary (two); squamous cell carcinoma of the lung (two); melanoma (one), transitional cell carcinoma of the bladder (one); and hepatocellular carcinoma (one). A > 50% decrease in colony formation occurred in 91% of human tumors tested at 10 μg/ml and in 86% tested at 1 μg/ml; a > 70% decrease in control colony formation occurred in 65% tested at 10 μg/ml and in 25% tested at 1 μg/ml. We conclude that (*a*) vitamins K_1 and K_3 have antineoplastic activity which is enhanced by combination with warfarin; and (*b*) vitamin K_3 has cytotoxic activity against human tumor explants in the soft agar assay system. [Cancer Treat Rep 69:527–532, 1985]

Vitamin K is a generic term for a homologous group of vitamins consisting of 1-methyl-1,4-naphthoquinone derivatives. The vitamin K quinone structure is essential for normal biosynthesis of several factors associated with hemostasis. The anticoagulant warfarin has a similar quinone-based structure as well. Recent trials in small cell lung cancer (1), osteogenic sarcoma (2), and adenocarcinoma of the colon (3) have suggested a beneficial effect associated with the use of warfarin in the treatment of malignancy. Rationale for the use of warfarin anticoagulation was originally based on animal studies which demonstrated that coagulation inhibition reduced the incidence of metastases (4). The report that high concentrations of warfarin could inhibit macromolecular synthesis in malignant cells (5) led us to initiate a series of investigations to evaluate the cytotoxicity of warfarin and the structurally related vitamin K compounds phylloquinone (vitamin K_1) and menadione (vitamin K_3). Original studies were performed using murine L1210 leukemia evaluated in a flask culture system (6). When the cytotoxic potential of vitamin K_3 was recognized, we evaluated the effects of vitamin K_3 on the formation of human tumor explant colonies in the soft agar (clonogenic) assay system.

MATERIALS AND METHODS

Drugs.—In the studies with L1210 cells, the following agents were tested: warfarin (Coumadin, DuPont, Wilmington, DE); vitamin K_1 (phytonadione, MSD-aqueous,

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colloidal solution of phylloquinone, Merck, Sharp & Dohme, West Point, PA); and vitamin K_3 (menadione sodium bisulfite, Sigma Corp, St. Louis, MO). The studies with HII4E hepatoma cells involved vitamin K_3. All experiments with menadione sodium bisulfite were carried out with a sodium bisulfite control. No effects of menadione sodium bisulfite could be attributed to the sodium bisulfite alone. The structural relationship of these agents is illustrated in figure 1. For studies involving human tumor explants, in addition to the vitamin K_3 tested in all patients, the following agents were tested in one or more instances: doxorubicin, 5-FU, methotrexate, mitomycin, vinblastine, cisplatin, carmustine, etoposide, and bleomycin. These agents were obtained commercially. For the vitamin K agents, the drug concentrations of 10 and 1 μg/ml were chosen to provide exposures in the range (approximately 10 μg/ml) recommended for the screening of new drugs for activity in the soft agar assay system (8,9). In addition, we have previously demonstrated that a 1-μg/ml concentration of vitamin K_3 is safely clinically achievable in man (10,11). The drug concentrations of standard chemotherapeutic agents were chosen based on pharmacokinetic information on clinically achievable levels in man (12). For studies involving vitamin K_3, all 34 human tumors were evaluated at 10 μg/ml, with 28 evaluated at a 1-μg/ml concentration. Drug concentrations used for the evaluation of the standard chemotherapeutic agents included cisplatin (0.01, 0.1, and 1 μg/ml); thiotepa (0.015, 5, and 50 μg/ml); vinblastine (0.1 and 1 μg/ml); 5-FU (50 and 100 μg/ml); etoposide (2 and 20 μg/ml); mitomycin (0.5 and 5 μg/ml); carmustine (0.3 and 3 μg/ml); and bleomycin (0.05, 0.5, and 5 μg/ml).

Flask culture technique.—We have previously established direct correlation of results obtained using the flask culture and the soft agar assay system for methotrexate with L1210 leukemia cells (7). Assessment of leukemic cell growth inhibition for vitamins K_1 and K_3 and warfarin in flask culture was done as previously described (7). Briefly, L1210 cell lines were maintained in vitro by serial passage in flask culture with cells grown in RPMI-1640 (Gibco) medium plus 15% inactivated fetal calf serum (Gibco) and were fed on 3- or 4-day intervals. For dose survival determination, cells were harvested in log phase growth after having been maintained by serial passage in 15-ml tissue culture flasks. Cells were incubated at the indicated concentration of drug, with cell counts made at 48, 72, 96, and 168 hours. Cultures were maintained at 37.5°C in an atmosphere containing 5% CO_2 and 95% air. Each assay was carried out in triplicate, with the results reported as the mean ± SD. Since only limited pharmacokinetic data on the disposition of vitamin K compounds in man were available, continuous drug exposure was used in most experiments to optimize conditions needed to observe cytotoxic effects. In addition to the continuous drug exposure used in most studies, a 24-hour period of drug exposure was employed in a number of experiments with L1210 cells. In these studies, after the 24-hour period of drug exposure, cells were washed by centrifuging flask contents at 200 × *g* for 10 minutes at room temperature, washing twice with fresh RPMI-1640 plus 15% fetal calf serum, then resuspending the cell button in 5 ml of fresh media and allowing the cells to grow for 4–7 days. At that time, cell counts were repeated.

Patient material.—Solid tumor specimens, malignant effusions, and/or ascites were collected from 34 patients undergoing procedures done as part of a diagnostic workup or as therapy for their disease. No major surgical procedures were performed solely to obtain specimens for drug sensitivity testing. Solid tumors or lymph nodes were immediately placed in CEM media (M. A. Bioproducts, Walkersville, MD) with 10% fetal calf serum under aseptic conditions in the operating room. The solid tumors were mechanically dissociated with scissors, incubated in 0.05% pronase, 0.02% collagenase, and 0.02% deoxyribonuclease at 37°C for 30 minutes, and immediately centrifuged and washed twice with media containing 15% fetal calf serum. The cell pellet was then further mechanically dissociated using scissors and scalpels. The resuspended cell pellet was then placed through a grid followed by passage through needles from 18 to 26 gauge. The resultant cell suspension was then layered on 9% Ficoll, 33.9% Hypaque, and 1.80 specific gravity and centrifuged at 5 × *g* for 20 minutes at 2000 rpm (13). Ascitic and pleural fluids were placed in sterile containers containing 100 units of preservative-free heparin/ml of malignant fluid. The volume was reduced by centrifuga-

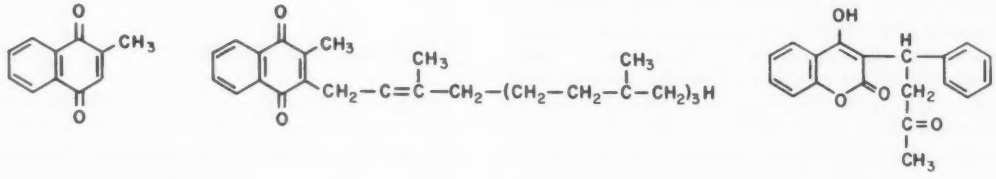

FIGURE 1.—Structures of vitamin K_3 (menadione), vitamin K_1 (phylloquinone), and warfarin.

tion and the fluids were then layered on the Ficoll-Hypaque directly. The cells at the blind interface following centrifugation were removed and washed free of Ficoll-Hypaque. The cell pellet was then checked to determine single cell suspension and viability of nucleated tumor cells by staining cells with trypan blue followed by microscopic review in a hemocytometer. Cell suspensions were then incubated with drug at the indicated concentrations and plated at an initial cell concentration of 0.5–1 × 6^6 cells/ml at 37°C, 95%–97% humidity, 5% CO_2, and 95% air. All plates were checked for single cell suspension before incubation.

Soft agar (clonogenic) assay.—The soft agar (clonogenic) assay was used to determine drug sensitivity on in vitro cell cultures from L1210 leukemia and HII4E hepatoma cells and from clinical solid tumors and effusions by a modification of the method of Hamburger and Salmon (14) as previously described (7). For clinical specimens, colonies (> 50 cells) usually appeared by Day 14 of culture, with plates read at between 14 and 28 days after plating. The number of colonies in control and drug-treated plates was determined by counting the colonies on an inverted-stage microscope. An average colony count of ⩾ 50 on the control plates was required to perform sensitivity analysis. Each plate was read and calculated as percent of colonies formed in control plates without drug. A variety of criteria have been arbitrarily used to define in vitro drug sensitivity in the soft agar assay system (15,16). In this report, results are reported in terms of 70% and 50% growth inhibition (percent decrease in colony formation) compared to control plates. All control and drug assays were done in triplicate, with results expressed as the mean ± SD.

RESULTS

The results of the dose-survival determinations for warfarin, vitamin K_1, and vitamin K_3 against murine L1210 cells in flask culture are seen in table 1. Complete growth inhibition as determined by cell counts performed at 96 hours occurred at concentrations of 200 μg/ml for warfarin, 75 μg/ml for vitamin K_1, and 4 μg/ml for vitamin K_3. The results of combined warfarin and vitamin K_1 and K_3 exposure on cell growth inhibition in the same system are illustrated in figure 2. Use of combined warfarin and vitamin K_1 and K_3 concentrations, which individually resulted in minimal growth inhibition, were associated with a more than additive combined cytotoxic effect. In the case of vitamin K_3, concentrations of 1 μg/ml in conjunction with warfarin at 70 μg/ml achieved nearly complete growth inhibition. Similar results were obtained when these experiments with combined warfarin and vitamins K_3 and K_1 were repeated using a 24-hour drug exposure period rather than continuous drug exposure. The cytotoxicity of the more active vitamin K_3 was evaluated in soft agar assay against murine leukemia L1210 and hepatoma HII4E; results are outlined in table 2. A > 70% decrease in colony formation was seen with vitamin K_3 at concentrations of 6.4 μg/ml for L1210 leukemia and 1 μg/ml for HII4E hepatoma lines. In addition, a clear dose-response relationship was observed.

TABLE 1.—Effects of warfarin and vitamins K_1 and K_3 against L1210 cells in flask culture*

% control growth inhibition	Dose (μg/ml) required to achieve inhibition		
	Vitamin K_3	Vitamin K_1	Warfarin
50	4	46	140
70	4	75	200

*Dose-survival assays in L1210. For dose-survival determinations, L1210 leukemia cells were harvested in log phase growth after having been maintained by serial passage in 15-ml tissue culture flasks. Cells at initial concentration of 1 × 10^5/ml were exposed continuously to indicated concentration of drug with cell counts made at 96 hrs.

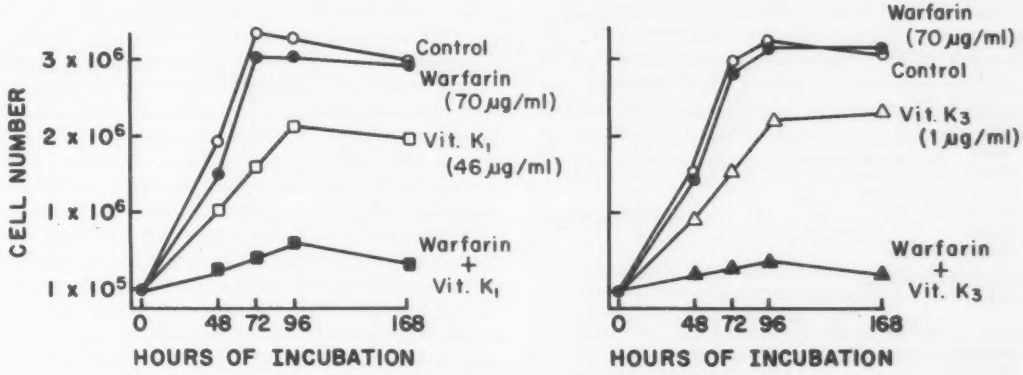

FIGURE 2.—Effect of warfarin and/or vitamins K_1 and K_3 on growth of L1210 leukemia cells. Cells in logarithmic growth at an initial concentration of 1 × 10^5/ml were exposed continuously to the indicated concentration of drug. Each point represents mean of 3 separate cultures at each time period. SDs were < 5% of the mean for each point.

TABLE 2.—Effects of vitamin K_3 on No. of tumor colonies against murine L1210 leukemia and HII4E hepatoma in soft agar assay

Vitamin K_3 concentration (μg/ml)	% of control colony	
	HII4E	L1210 leukemia
64	7	11
46	8	12
10	10	13
6.4	11	17
4.6	11	55
2.8	11	98
1.0	12	98
0.5	31	99
0.1	88	99

Based on the effects of vitamin K_3 on the L1210 system, we evaluated the cytotoxic potential of this agent in 34 human tumor explants using the clonogenic soft agar system. In addition to evaluating vitamin K_3, human tumor specimens were also tested against a mean of 4.2 standard chemotherapeutic agents which the patients had not yet received; selection of standard drugs tested was based on clinical assessment of drugs most likely to have activity. Primary sites of human tumors evaluated included adenocarcinoma of the breast (16 patients), ovary (five), colon (two), stomach (two), kidney (two), and unknown primary (two); squamous cell carcinoma of the lung (two); and transitional cell carcinoma of the bladder, melanoma, and hepatocellular carcinoma (one each).

The dose-survival assay results for the 34 human tumor explants tested against vitamin K_3 are shown in table 3 for both 10- and 1-μg/ml concentrations. Vitamin K_3 at the 10-μg/ml concentration resulted in a > 50% inhibition of control colony formation in 31 of 34 (91%) evaluated tumors; at the 1-μg/ml concentration, a > 50% inhibition of control colony formation was seen in 24 of 28 patients (86%). A > 70% inhibition of control colony formation was seen in 65% of patients at the 10-μg/ml concentration and in 25% of the patients at the 1-μg/ml concentration of vitamin K_3. For patients with breast cancer, 15 of 16 (93%) showed a > 50% inhibition of control colony formation when exposed to 10 μg/ml of vitamin K_3. The activity against ovarian and renal cell carcinoma also was noteworthy. The dose-response characteristics for vitamin K_3 cytotoxicity against the human tumor explants in the soft agar system are shown in figure 3. In almost all patients, higher vitamin K_3 dosage was associated with a greater degree of cytotoxicity. No cases of tumor growth enhancement with vitamin K_3 were seen. For comparison, the dose-survival assay results for vitamin K_3 can be compared to those for the most active of the standard chemotherapeutic agents evaluated in these same tumor explants. For doxorubicin, methotrexate, and vinblastine, a > 70% inhibition of control colony formation was seen at the highest concentration tested in 38%, 36%, and 28% of tumor tested, respectively.

DISCUSSION

The studies reported here indicate that warfarin and especially vitamins K_1 and K_3 have antineoplastic activity against murine malignant cell growth. In addition, our data indicate that vitamin K_3 is a potent inhibitor of human tumor colony formation in the soft agar assay system. Since preliminary data from the National Cancer Institute and others support the concept of using the human tumor clonogenic assay for new drug screening (8,9), our in vitro results suggest potential clinical activity for vitamin K_3 in patients with cancer.

Information from other investigators supports the concept that the vitamin K series are cytotoxic agents for malignant cells. In earlier work, vitamin K_1 has been shown to inhibit macromolecular synthesis in mouse leu-

TABLE 3.—Effects of vitamin K_3 on human tumor colony formation

Tumor type	Vitamin K_3 (10 μg/ml)			Vitamin K_3 (1 μg/ml)		
	No. of patients tested	% decrease in colony formation		No. of patients tested	% decrease in colony formation	
		50	70		50	70
Adenocarcinoma						
Breast	16	15	9	13	12	4
Ovary	5	4	4	3	2	1
Colon	2	2	1	1	1	0
Stomach	2	2	1	2	2	1
Kidney	2	2	2	2	2	0
Unknown primary	2	1	1	2	0	0
Squamous cell carcinoma						
Lung	2	2	2	2	2	0
Other						
Bladder	1	1	0	1	1	0
Melanoma	1	1	1	1	1	0
Hepatocellular	1	1	1	1	1	1
Total	34	31 (91%)	22 (65%)	28	24 (86%)	7 (25%)

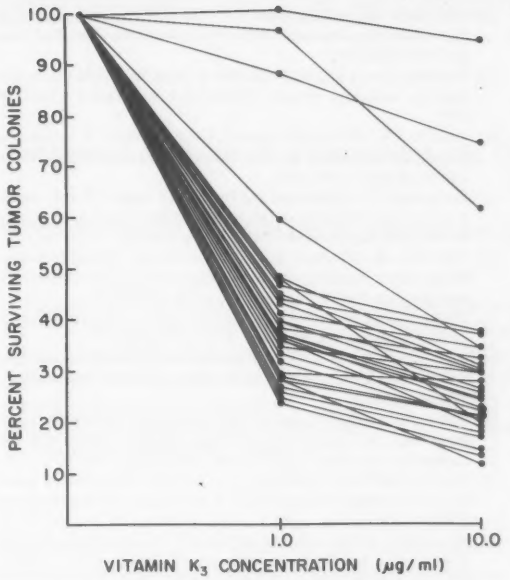

FIGURE 3.—Dose-response effect of vitamin K_3 (menadione) on the formation of tumor colonies from human tumor explants. Explants were exposed continuously to the indicated concentration of vitamin K_3 in a soft agar assay system. Results are reported in terms of % decrease in colony formation compared to control plates.

kemia, HeLa, and Ehrlich ascites carcinoma cells (17-19), but not in nonmalignant cell lines. In our studies, vitamin K_3 was approximately 25 times more cytotoxic than vitamin K_1. Other in vitro studies with a human neuroblastoma cell line have shown equally impressive cytotoxic effects with vitamin K_3 as compared to vitamin K_1 (20).

The compound menadiol sodium diphosphate (Synkavite) is a commercially available vitamin K preparation which is metabolized in vivo to vitamin K_3 and provides a means of administration of vitamin K_3 to man (21). Following administration of 20 mg/m^2 of menadiol sodium diphosphate im to cancer patients, we have observed vitamin K_3 levels as high as 1.8 μg/ml (10,11). This circulatory concentration of vitamin K_3 (safely achieved in man) exceeds the concentration associated with in vitro cytotoxicity of human tumor explants in the soft agar assay system. It should be noted that menadiol sodium diphosphate has a Food and Drug Administration-approved indication in man calling for a dosage level of 75 mg iv in the assessment of liver function. Furthermore, the use of menadiol sodium diphosphate in such dosage given as an addition to radiation therapy in two randomized clinical trials has been associated with significant survival benefit in both non-small cell lung carcinoma and oropharyngeal carcinoma populations (22,23). Whether such survival benefit resulted from radiation potentiation or a direct cytotoxic effect of vitamin K_3 remains to be determined.

Warfarin has important influences on the metabolism of vitamin K compounds by interacting with enzyme systems responsible for vitamin K degradation (21). As a result, the enhanced cytotoxicity seen in vitro with combined warfarin and vitamin K use against L1210 leukemia cells could reflect changes in the exposure of cells to metabolites of vitamin K. Although a separate line of evidence supports the ongoing interest in anticoagulants as antimetastatic agents (1-4), the cytotoxic effects of vitamin K_3 seem independent of effects on the coagulation mechanism since vitamin K_3 is not an important antagonist to warfarin anticoagulation (21,24).

The mechanism by which vitamin K acts as an antineoplastic agent is currently unknown. However, vitamin K compounds have a quinone structure and the cytotoxicity of other quinones is thought to be mediated through their electron reduction to semiquinone radicals which interact with molecular oxygen to produce injury (25). Changes in calcium metabolism, glutathione pool sizes, and cell membrane properties (26-29) have also been associated with vitamin K use in a number of in vitro systems. In addition, we have obtained preliminary evidence that vitamin K may affect salvage pathway synthesis of pyrimidine nucleotides necessary for DNA synthesis (6,30). At cytostatic concentrations of warfarin and vitamin K_3, marked inhibition of salvage pathway function occurred (as assessed by incorporation of ^{14}C-labeled deoxythymidine), while little influence on de novo pathway function was seen (as assessed by the incorporation of tritiated deoxyuridine). Finally, the selective distribution of vitamin K agents to tumor-bearing regions has been explored using radiolabeled preparations of sodium diphosphate of high specific activity as radioactive anticancer agents (31,32). Further studies will be required to more precisely define the mechanism or mechanisms underlying the vitamin K_3 cytotoxicity identified in the present studies.

In summary, our in vitro studies indicate that (*a*) vitamins K_1 and K_3 have antineoplastic activity which is enhanced by combination with warfarin; and (*b*) vitamin K_3 has cytotoxic activity against human tumor explants in the soft agar assay system. Clinical studies evaluating vitamin K_3 and possibly other vitamin K analogs as potential antitumor agents can be recommended.

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Antitumor Activity of a Benzaldehyde Derivative[1]

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Benzaldehyde, in the form of 4,6-benzylidene-α-D-glucose (BG), was given iv at a daily dose of 720–1800 mg/m² to 65 patients with inoperable carcinoma in the advanced stages. The overall objective response rate was 55%; seven patients achieved complete response, 29 achieved partial response, 24 remained stable, and five showed progressive disease. Response was seen in various cell types. Prolongation of survival was apparent for the patients. Toxic reactions were not observed during long-term injection with BG. [Cancer Treat Rep 69:533–537, 1985]

We have previously shown that a volatile fraction of figs absorbed by active charcoal has activity against transplanted tumors in mice and also against human tumors (1). An effective agent in the volatile fraction of figs was found to be benzaldehyde (2). We then reported clinical trials with β-cyclodextrin benzaldehyde inclusion compound (CDBA) (3).

This report summarizes our experience with another type of benzaldehyde derivative, 4,6-benzylidene-α-D-glucose (BG), in patients with carcinoma in the advanced stages on the basis of animal experiments (4).

MATERIALS AND METHODS

Sixty-five patients with carcinoma of varied histologic types in the advanced stages were treated, including 36 men and 29 women with a mean age of 58 years (range, 28–87). No exclusions were made for inadequate trial, early death, or cases that could not be evaluated. The extent of disease was carefully defined by clinical measurement as well as roentgenographic, computed tomographic, scintigraphic, ultrasonographic, endoscopic, and angiographic study. Routine hematologic and biochemical examinations were performed twice a month. Baseline studies included physical examination, measurement of body weight and height, cbc, blood urea nitrogen, creatinine, bilirubin, alkaline phosphatase, AST, uric acid, serum protein, albumin, urinalysis, electrocardiogram, CEA, alpha-fetoprotein, and measurement of tumor size. Chest x-ray for lung and esophageal cancer, endoscopy for gastric carcinoma, and computed tomography for brain and liver tumors were made about once a month.

Functional performance status at the onset of BG therapy was evaluated according to the criteria of the Japanese Ministry of Welfare as follows: *grade 0*—ambulatory (none of the patients); *grade 1*—light housework only (two); *grade 2*—bedridden < 50% of the time (16); and *grade 3*—bedridden > 50% of the time (47). Sixty-one of 65 patients had had prior treatment: 60 had received chemotherapy, 25 had undergone surgery, nine had received radiotherapy, and one had had an embolization. No known curative treatment existed for these patients, and there was no palliative treatment that we thought would benefit them. Informed consent was obtained in each case.

Evaluation of response to therapy was based on the criteria of the Japanese Ministry of Welfare, concerning the chemotherapeutic effects on solid tumors. Tumor measurements were recorded at least every month. Criteria for responses were as follows: *complete response (CR)*—the disappearance of all clinical evidence based on the measurable lesion, evaluable lesion, and tumor, and the absence of appearance of new lesions for > 4 weeks; *partial response (PR)*—a > 50% decrease in the product of the perpendicular diameters of the lesion without the appearance of new lesions for > 4 weeks, or a > 30% decrease in one-dimensionally measurable lesions, no aggravation of all clinical evidence caused by evaluable lesions and tumor, and the absence of appearance of new lesions for > 4 weeks; *no change*—a < 50% decrease in two-dimensionally measurable lesions, a 30% decrease in one-dimensionally measurable lesions, or a ⩽ 25% in-

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TABLE 1.—Description of the complete and partial responders

Patient No.	Primary site	Histology*	Prior therapy†	Extent of disease‡	Performance status	Dose (mg/m^2)	Response§	Response duration (mos)	Responding sites
1	Tongue	SC	Radio; chm	Tongue;¶ palatum;¶ Lung	3	720	PR	5.0	Tongue; palatum; lung
2	Tongue	SC	Chm	Tongue¶	3	1800	NC		
3	Tongue	SC	Chm	Tongue¶	3	1800	NC		
4	Tongue	SC	Sg; radio; chm	Tongue; orbita;¶ lung¶	3	1800	NC		
5	Retropharynx	ML	Chm	Retropharynx;¶ trachea¶	2	900–1800	CR	27.0	Retropharynx; trachea
6	Pharynx	SC	Radio; chm	Pharynx; neck;¶ lumbar vertebra¶	3	1800	PR	9.0	Neck
7	Skin of face	SC	Sg; chm	Skin of face¶	2	1800	PD		
8	Lymph node of neck	ML	Chm	Lymph node of neck¶	1	1800	CR	20.0	Cervical lymph node
9	Spinal cord (cervical area)	Glioma		Spinal cord¶ (cervical area)	3	1800	PR	2.0	Spinal cord (cervical area)
10	Esophagus	SC	Chm	Esophagus¶	3	900	PR	5.0	Esophagus
11	Lung	AC	Chm	Lung¶	3	720	NC		
12	Lung	SC	Chm	Lung¶	2	720	CR	4.0	Lung
13	Lung	Large cell carcinoma	Chm	Lung;¶ effusion	3	1800	CR	4.0	Lung
14	Lung	AC	Sg; chm	Lung;¶ effusion¶	2	1800	PR	10.0	Lung; effusion
15	Lung	Small cell carcinoma	Chm	Lung;¶	3	1800	PR	2.0	Lung
16	Lung	Small cell carcinoma	Chm	Lung;¶ effusion¶	3	1800	NC		
17	Lung	SC	Sg; chm	Lung¶	3	1800	NC		
18	Lung	SC	Sg; chm	Lung¶	3	1800	NC		
19	Lung	Sarcoma	Chm	Lung;¶ lumbar vertebra¶	3	1800	PD		
20	Lung	Large cell carcinoma	Chm	Lung;¶ effusion¶	3	1800	NC		
21	Lung	SC	Chm	Lung;¶ effusion¶	3	1800	NC		
22	Breast	AC	Sg; radio; chm	Breast; skin;¶ lung¶	3	1800	PR	4.0	Skin
23	Breast	AC	Sg; radio; chm	Breast; lung;¶ effusion;¶ neck (lymph node)¶	3	1800	PR	3.0	Lung; neck (lymph node)

24	Breast	AC	Sg; radio; chm	Breast; lung;¶ effusion¶	3	1800	NC		
25	Stomach	AC	Sg; chm	Stomach; liver¶	2	720	CR	1.5	Liver (stomach resected)
26	Stomach	AC	Chm	Stomach;¶ PC	3	720	NC		
27	Stomach	AC		Stomach¶	3	900–1800	PR	24.0	Stomach
28	Stomach	AC	Chm	Stomach¶	3	1800	PR	3.0	Stomach
29	Stomach	AC	Sg; chm	Stomach; PC¶	3	1800	PR	3.5	Peritoneum
30	Stomach	AC	Chm	Stomach;¶ PC	3	1800	PR	7.0	Stomach
31	Stomach	AC	Chm	Stomach;¶ PC; cervical vertebra¶	3	1800	NC		
32	Stomach	AC	Chm; sg	Stomach; liver¶	2	1800	PR	2.0	Liver
33	Stomach	AC	Sg; chm	Stomach; peritoneum¶	2	1800	NC		
34	Stomach	AC	Sg; chm	Stomach;¶ PC	3	1800	NC		
35	Stomach	AC	Sg; chm	Stomach; PC;¶ kidney;¶ uterus¶	3	1800	PR	4.0	Peritoneum
36	Stomach	AC	Chm	Stomach;¶ PC	3	1800	NC		
37	Liver	Hepatoma	Chm	Liver¶	3	1800	NC		
38	Liver	Hepatoma	Embolization; chm	Liver¶	3	900–1800	PR	26.0	Liver
39	Liver	Hepatoma	Chm	Liver¶	3	1800	PD		
40	Bile duct	AC	Sg; chm	Bile duct¶	3	900	PR	5.5	Bile duct
41	Bile duct	AC	Sg; chm	Bile duct¶	3	900	NC		
42	Gallbladder	AC	Sg; chm	Gallbladder; liver¶	3	720	PR	7.0	Liver
43	Pancreas	AC	Chm	Pancreas;¶ lung¶	3	1800	PR	5.0	Pancreas
44	Pancreas	AC	Chm	Pancreas;¶ lung¶	3	1800	PD		
45	Colon	AC	Chm	Colon;¶ PC	3	720	NC		
46	Colon	AC	Sg; chm	Colon; lung¶	2	720	NC		
47	Colon	AC	Sg; chm	Colon; lung¶	2	720	CR	11.0	Lung
48	Colon	AC	Sg; chm	Colon; liver¶	3	720	NC		
49	Colon	AC	Chm	Colon¶	3	900	PR	5.5	Colon

Continued

TABLE 1.—*Continued*

Patient No.	Primary site	Histology*	Prior therapy†	Extent of disease‡	Performance status	Dose (mg/m²)	Response§	Response duration (mos)	Responding sites
50	Colon	AC	Chm	Colon;¶ PC	3	900	PR	10.0	Colon
51	Colon	AC		Colon¶	3	1800	CR	4.5	Colon
52	Colon	AC	Sg; chm	Colon; brain;¶ liver¶	2	1800	PR	1.5	Brain; liver
53	Colon	AC	Sg; chm	Colon; lung¶	1	1800	NC		
54	Colon	AC	Chm	Colon;¶ liver;¶ peritoneum	3	1800	PR	1.5	Liver
55	Colon	AC	Sg; chm	Colon; brain;¶ liver;¶ peritoneum	3	1800	PR	4.5	Brain; liver
56	Colon	AC	Sg; chm	Colon;¶ PC	3	1800	PR	5.5	Colon
57	Rectum	AC	Sg	Rectum liver;¶ pelvis¶	2	720	PR	11.5	Liver; pelvis
58	Rectum	AC	Sg; chm	Rectum; liver¶	3	1800	PR	1.0	Liver
59	Rectum	AC	Chm	Rectum;¶ liver;¶ pelvis	3	1800	PR	12.0	Rectum; liver
60	Rectum	AC	Sg; chm	Rectum; lung;¶ liver¶	3	1800	PD		
61	Rectum	AC	Sg; chm	Rectum; lung;¶ kidney;¶ sacrum¶	3	1800	NC		
62	Uterus	SC	Radio; chm	Uterus;¶ pelvis¶	2	1800	PR	18.0	Uterus; pelvis
63	Uterus	SC	Radio; chm	Uterus;¶ pelvis¶	3	1800	PR	2.0	Pelvis
64	Uterus	SC	Radio; chm	Uterus;¶ pelvis;¶ rectum¶	3	1800	NC		
65	Urinary bladder	Transitional cell carcinoma	Chm	Urinary bladder¶	3				

*SC = squamous cell carcinoma; ML = malignant lymphoma; AC= adenocarcinoma.
†Radio = radiotherapy; chm = chemotherapy; sg = surgery.
‡PC = peritonitis carcinomatosis.
§NC = no change; PD = progressive disease.
¶Evaluable site.

crease in both lesions, no aggravation of secondary lesions, and the absence of new lesions for > 4 weeks; and *progressive disease*—either a 25% increase in tumor size from the original measurement or the appearance of new lesions.

BG was dissolved in buffered physiologic saline solution (pH 7.5) to make a 1% solution before use. Because of the low solubility of BG, 1% solution is reached almost at the saturation point. BG solution can be administered through either an iv, im, or sc injection. In most cases, BG was given to the patients by iv injection at dosages of 720–1800 mg/m^2/day through an iv drip method within 30 minutes. In only one patient with primary hepatoma, BG was intra-arterially injected at a daily dose of 36 mg/m^2 for 10 days prior to an iv drip administration.

RESULTS

Descriptions of the responders are detailed in table 1. The overall objective response rate was 55.3% (complete response, 10.7%; partial response, 44.6%). The patients were treated daily until response occurred. Once a response occurred, the treatment continued daily with 1800 mg/m^2 of BG given as maintenance therapy. The longest response duration was demonstrated in the patient with malignant lymphoma. His response has lasted 27 months in normal condition so far. No striking differences in response rates among the various cancers were apparent. Following BG treatment, cancer cells exhibited degenerative change as with CDBA therapy. Peculiar pearl formation in squamous cell carcinoma and pleomorphic cells in adenocarcinoma or in malignant lymphoma were observed. Toxic reactions were not observed during long-term injection with BG. There were no apparent sex differences in response rate.

DISCUSSION

The present protocol was designed to use BG for the treatment of patients with varied cell types of carcinoma in the advanced stages. Although classic antitumor therapy had been abandoned in all these patients, clinical improvement with CR and PR was evident. The dosage of BG was gradually increased to 1800 mg/m^2/day, the daily clinical dosage that we are now temporarily using, since the use of 50 mg/kg (1800 mg/m^2) showed dose-dependent inhibition against Walker 256 tumors in rats (4).

Pettersen et al (5) reported that BG inhibited protein synthesis in NHIK 3025 cells without indicating any specific mitotic inhibition. Sakaguchi et al (6) also reported benzaldehyde influence upon the cell membrane protein such as fibronectin. However, the mechanism of action of antitumor activity observed in the BG treatment is not yet clear. In addition, the daily dosage of BG in this study is neither firmly established nor conclusive. Further evaluation of the effect of this drug on the patients with cancer will be necessary before we can assess its efficacy.

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Treatment of Advanced Endometrial Carcinoma With Doxorubicin and Cisplatin: Effects on Both Untreated and Previously Treated Patients[1,2]

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Sixteen patients with advanced (International Federation of Gynecology and Obstetrics stage III and IV) adenocarcinoma of the endometrium were treated with twelve 28-day cycles of doxorubicin and cisplatin. Response was achieved in 92% of patients (11 responses among 12 patients) who had received no prior chemotherapy and in 50% (two responses among four patients) of previously treated patients. Median survival was 10 months. Doxorubicin and cisplatin were readily administered on an outpatient basis with comparatively low major toxic effects, primarily hematologic, renal, and gastrointestinal. These results indicate that doxorubicin and cisplatin combination therapy is effective with acceptable toxicity in patients with advanced endometrial carcinoma. [Cancer Treat Rep 69:539–542, 1985]

Adenocarcinoma of the endometrium remains the most common gynecologic malignancy. It is predominantly a disease of perimenopausal and postmenopausal age groups since > 75% of all cases occur after 40 years of age. At diagnosis, most women have localized disease, with 5-year survival rates for patients with stage I disease reported to be as high as 87% (1,2). Approximately 10% of these patients with advanced disease, however, have more extensive spread with markedly shortened survival (3,4). As a result, chemotherapy has been employed with increasing frequency.

Doxorubicin and cisplatin has proven to be a particularly efficacious combination for gynecologic cancers, particularly ovarian cancer (5). Based on the combination's effects against ovarian cancer and the activity of the agents when used singly in endometrial carcinoma, a trial using doxorubicin and cisplatin was undertaken in patients with endometrial carcinoma (6,7). Reported herein are the results of the administration of doxorubicin and cisplatin in combination for both untreated and previously treated patients with stage III and IV endometrial carcinoma.

PATIENTS AND METHODS

Patient Selection

Between August 1979 and April 1982, 16 patients with stage III and IV endometrial carcinoma were placed in a study protocol at The New York Hospital-Cornell Medical Center. Both untreated and previously treated patients were eligible for the protocol if they had (*a*) histologically proven stage III or IV adenocarcinoma of the endometrium, (*b*) measurable disease, (*c*) an expected survival of > 1 month, (*d*) serum creatinine < 1.5 mg/dl, creatinine clearance 50% of predicted value, (*e*) wbc count > 4000/mm^3 and platelet count > 100,000/mm^3, (*f*) bilirubin < 1.5 times normal, (*g*) no significant cardiac disease, and (*h*) no prior treatment with cisplatin and/or doxorubicin.

Treatment Dosage and Schedule

During the 4 hours preceding treatment, patients were given 1–2 L of iv fluid (usually 5% dextrose in 0.45% saline). When urine output increased to > 125 ml/hour,

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doxorubicin (60 mg/m^2) was given over 30 minutes. This was followed by iv furosemide (20–40 mg) as needed to ensure adequate urine output. Cisplatin (60 mg/m^2) was then infused over 1 hour. Urine output was maintained at > 100 ml/hour for at least 4 hours after cisplatin was given, using iv furosemide when necessary. The treatment was administered every 28 days. Doses of doxorubicin and cisplatin were modified as has previously been described (5).

Response Evaluation

Criteria for progressive disease, no change, and partial response (PR; a 50% reduction in tumor) have been previously described (5). Complete clinical response (CCR) was defined as the clinical disappearance of all physical and radiologic (including sonographic and/or computerized tomographic) evidence of tumor. In patients completing 12 cycles of chemotherapy who were in CCR, a "second-look" laparotomy, as has been described, was encouraged to document the absence of disease, a complete pathologic response (CPR) (5).

Toxicity Evaluation

All patients were evaluated systematically for toxicity. The major toxic effects were gastrointestinal, myelosuppressive, neurologic, and renal. Toxicity was graded from 0 (no toxicity) to 4 (severe, life-threatening), as has been described (5).

RESULTS

Response

The response frequency for all patients treated with doxorubicin and cisplatin (CPR, CCR, and PR) was 82% (table 1). Six patients achieved a CCR. Two of these six patients had second-look laparotomy. Both patients were found to have persistent disease, were re-treated, but subsequently failed chemotherapy and died. Of the four other patients in CCR, two are still receiving treatment (entering the 8th and 12th months) and doing well, one refused therapy and later died, and one was deemed unsuitable for a second-look laparotomy for medical reasons and remains in remission 16 months after discontinuance of therapy. Seven female patients (44%) achieved PR. Of these seven women, five have died and two are receiving alternate therapy. Of the three patients not responding to initial therapy, one patient had no change in disease and later died, while two other patients had progressive disease, both dying due to advancing disease.

Survival

Median survival calculated from the time of institution of doxorubicin and cisplatin was 10 months for the entire group of patients, 12 months for responders, and 3 months for nonresponders (table 1).

Variables

Stage.—There were six patients with stage III disease and ten with stage IV disease. Response frequency did not differ significantly with stage. Five of the six patients (83%) with stage III carcinoma had a response, with one CCR, whereas eight of ten patients (80%) with stage IV carcinoma responded. Median survival in both groups was almost identical. Patients with stage III disease had a median survival of 11 months, while those with stage IV disease had a median survival of 10 months (table 1).

Prior therapy.—Patients who had had no previous chemotherapy responded better (92% response) than those who had had previous chemotherapy (50% response). All CCRs occurred only in those patients with no prior treatment. Although the number of previously treated patients was small, patients who had received prior chemotherapy had a median survival of 8 months, whereas patients who had received no prior chemotherapy had a median survival of 11 months (table 1).

Age.—Response frequency was not related to age (table

TABLE 1.—Response by variables

	Total No. of patients	Response*					Response rate (%)	Median survival (mos)
		CPR	CCR	PR	NC	PD		
Total	16	0	6	7	1	2	82	10
Stage								
III	6	0	1	4	0	1	83	11
IV	10	0	5	3	1	1	80	10
Previous chemotherapy								
Yes	4	0	0	2	1	1	50	8
No	12	0	6	5	0	1	92	11
Age (yrs)								
< 60	4	0	0	3	0	1	75	11
> 60	12	0	6	4	1	1	83	9

*NC = no change; and PD = progressive disease.

1). Of the four women < 60 years of age, there were three patients who responded to therapy (75%), with a median survival of 11 months. Of the 12 older patients, ten responded favorably (83%).

Toxicity.—Toxicity was predominantly gastrointestinal, hematologic, and renal (table 2).

Gastrointestinal.—All patients had vomiting and nausea on the day of cisplatin administration (grade 2), which was only minimally relieved with antiemetics. One patient experienced a gastrointestinal bleed (grade 3).

Hematologic.—Six patients experienced leukopenia: three, grade 4; two, grade 2; and one, grade 1. All secondary infections were successfully treated with antibiotics. Thrombocytopenia occurred in five patients; three had platelet counts < 20,000/mm^3 (grade 4). There was no significant bleeding resulting from thrombocytopenia.

Nephrotoxicity.—Four patients had diminution of their creatinine clearance. Three patients had experienced mild depression (grade 1). Only one patient had a creatinine clearance < 75% of pretreatment values. No treatment was delayed > 1 month.

Neurotoxicity.—Two patients experienced peripheral neuropathy; both were found to have mild muscular weakness (grade 2).

Other toxic effects.—One patient developed mild ototoxicity. On testing, most of her hearing was outside the normal human range. Three patients experienced doxorubicin extravasation. Two patients had grade 1 and only one patient had grade 2 extravasation. Congestive heart failure was experienced by two patients. One patient had grade 2 symptoms which may not have been related to the doxorubicin. The second patient had severe grade 4 symptoms. The patient, however, died shortly from advancing disease after refusing further therapy.

DISCUSSION

Doxorubicin and cisplatin each has documented efficacy in endometrial carcinoma when used alone. In this study they were combined to produce an 82% response rate in a group of previously treated and untreated patients. This response is comparable or perhaps superior to other regimens reported in similar groups of patients (8–11).

TABLE 2.—Toxicity

Toxicity	Total No. of patients	Grade—			
		1	2	3	4
Gastrointestinal	16	0	15	1	0
Hematologic					
Leukopenia	6	1	2	0	3
Thrombocytopenia	5	0	0	2	3
Renal	4	3	1	0	0
Neurologic	2	0	2	0	0
Hearing	1	0	1	0	0
Extravasation	3	2	1	0	0
Cardiac	2	0	1	0	1

Deppe et al (8) reported a response rate of 45% (13 responses among 29 patients) with cyclophosphamide, doxorubicin, 5-FU, and megestrol acetate. Of the 13 responders, only eight (28%) had CCRs. The overall response rate in our study was higher (82% vs 45%), and the CCR rate (35% vs 28%) was also higher. In addition, the regimen of Deppe et al (8) had substantially more toxicity.

Cohen et al (11), using melphalan, 5-FU, and medroxyprogesterone acetate, achieved an 86% objective response rate (six responses among seven patients). There were five PRs and one CR (14%). The patients were subject to significant toxicity. Most of these patients (four) developed severe leukopenia (wbc count < 1000/mm^3), and two of these patients had to be hospitalized for infection.

Seski et al (9), using doxorubicin and cyclophosphamide, reported a 31% response rate (eight responses among 26 patients). All eight responses were PRs; no CRs were achieved.

The combination of doxorubicin, cyclophosphamide, and cisplatin administered by Koretz et al (10) on both previously treated and untreated patients with advanced endometrial carcinoma achieved a response rate of 56% (four responses among seven patients). All four responses were only PRs, with a median response duration of only 2 months. Our data, using two of the three drugs (excluding cyclophosphamide), are superior. Although there may be a variety of explanations, such as patient selection, it is quite possible that use of two drugs at a more effective dosage may be superior to reduction of the dosage of two drugs and inclusion of a third drug which, while active, may be inferior. Indeed, results such as these necessitate the careful evaluation of adding an ever-increasing number of drugs in combination based on theoretical advantage alone.

While our data and those referenced give cause for optimism, it should be emphasized that meaningful, long life and/or cure demand the achievement of a CPR determined by a second-look re-evaluation by surgery. The utility of this surgery in ovarian cancer has now been well-established and such surgery should prove to be of value in endometrial cancer once further refinements and effectiveness in treatment are achieved (5).

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Eastern Cooperative Oncology Group Phase II Studies in Advanced Measurable Colorectal Cancer. I. Razoxane, Yoshi-864, Piperazinedione, and Lomustine[1,2]

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During a 6-month interval, Eastern Cooperative Oncology Group randomized 127 patients who had received prior chemotherapy, and who had advanced measurable, surgically incurable colorectal cancer, to receive piperazinedione (PZD), Yoshi-864, or razoxane (ICRF-159). The observed response (and median survival) rates were: PZD, one of 35 patients (17 weeks); Yoshi-864, one of 34 (19 weeks), and ICRF-159, none of 38 (23 weeks). Among 107 evaluable patients, there were five episodes of life-threatening toxicity with PZD (one death) and four with ICRF-159 (two deaths). In the same protocol, 42 evaluable patients who had not received prior chemotherapy were randomized to be treated with lomustine (CCNU) or one of the three drugs in the "previously treated" trial. One CR (41 weeks) was seen with ICRF-159 and two PRs were seen with CCNU. Life-threatening toxicity occurred in three patients, two who received CCNU (one death) and one who received PZD. No survival advantage was seen. We do not encourage further phase II trials in colorectal cancer with the agents studied. [Cancer Treat Rep 69:543–545, 1985]

The failure of chemotherapy regimens composed of multiple drugs to achieve a significant response rate in advanced metastatic colorectal cancer has encouraged further trials of phase II drugs. No drug trial by the Eastern Cooperative Oncology Group (ECOG) has yielded results significantly superior to treatment with 5-FU alone. Trials by ECOG in colorectal cancer have demonstrated the importance of including patients who have had no previous exposure to chemotherapeutic agents (1–3).

METHODS

EST 1277 was a randomized prospective trial designed to evaluate several drugs (piperazinedione, Yoshi-864, razoxane, methotrexate, melphalan, dactinomycin and lomustine) against histologically confirmed, advanced measurable, metastatic, or recurrent nonresectable adenocarcinoma of the colon and rectum. Four of those agents form the basis of this report: razoxane (ICRF-159), Yoshi-864, piperazinedione (PZD), and lomustine (CCNU) [included because of activity in a previous trial (4)]. Measurable tumor was defined as a clearly measurable mass (by ruler or calipers) upon physical examination or chest x-ray. Malignant hepatomegaly was acceptable when the liver contained biopsy-proven cancer and the edge extended 5 cm below the costal margin or the xiphoid process. The liver scan could only be used as an

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indicator when there was a clearly defined measurable profusion defect of at least 5 cm in diameter. Computerized tomographic scans, ultrasounds, and CEAs were not considered measurable disease according to this protocol. An expected survival of at least 2 months and a patient who was not totally bedridden were required. Minimum biochemical requirements included: BUN ≤ 25 mg/100 ml, creatinine ≤ 1.5 mg/100 ml, bilirubin ≤ 2 mg/100 ml, wbc count ≥ 4000/mm^3, platelet count ≥ 100,000/mm^3, and hemoglobin ≥ 10 g/100 ml. Treatment could not be instituted within 3 weeks of a bowel resection or bypass, or within 2 weeks of a laparotomy. Elevated wbc counts or fever required evaluation to rule out occult infection. For those patients who had previous chemotherapy (prior), a 28-day delay was required (7 weeks if the previous chemotherapy was a nitrosourea). The indicator of measurement could not be in an area of prior radiation therapy. If irradiated to the pelvis, a 33% dose reduction was mandated for the first course. Since the objective of this study was to search for drugs that might be useful in advanced colorectal cancer, and not to compare one drug to another, randomization was utilized as a means of stratifying unstratified prognostic variables among the treatment arms. The randomizations for previously untreated patients were unbalanced for CCNU and ICRF-159.

The following dose regimens were utilized: *ICRF-159*—1 g/m^2 orally in divided doses every 8 hours daily × 3 days every 3 weeks; *Yoshi-864*—74 mg/m^2 iv daily × 5 every 6 weeks; *PZD*—12 mg/m^2 iv every 3 weeks; and *CCNU*—130 mg/m^2 orally every 6 weeks (previously untreated patients only).

Upon treatment failure, patients who still met criteria for initial entry could be randomized to ICRF-159, Yoshi-864, or PZD. Dose reductions based on nadir counts were: 25% for wbc counts of 2500-3999/mm^3 or platelet counts of 75,000-149,999/mm^3, and 50% for wbc counts of 1500-2499/mm^3 or platelet counts of 40,000-74,999/mm^3, with treatment discontinued for lower nadirs. Yoshi-864 was discontinued for central nervous system toxicity and PZD was postponed for prolongation of prothrombin or partial thromboplastin times.

Objective tumor response was graded according to ECOG criteria. Complete response (CR) was defined as a complete disappearance of all clinically detectable tumor. Partial response (PR) was defined as a reduction of at least 50% of the product of the longest perpendicular diameters of the most clearly measurable mass lesion with no progression of any other lesion. When hepatomegaly was the indicator, there had to be a 30% reduction in the sum of the liver measurements below the costal margin accompanied by a trend toward normalization of abnormalities of liver function. In addition, responding patients could not have new areas of malignant disease found during the period of response. There could be no significant deterioration in weight (10%) or loss of performance status (ECOG score of 1).

RESULTS AND DISCUSSION

Toxic effects and responses are summarized in tables 1 and 2. ICRF-159 was associated with anemia that appeared to be drug-related in four previously treated patients and two previously untreated patients. PZD's major toxicity was thrombocytopenia, which tended to be prolonged, requiring delay before re-treatment. Neurologic symptoms occurred in five previously treated patients and one previously untreated patient who received Yoshi-864. Gastrointestinal toxicity occurred in two previously treated patients and one previously untreated patient. Skin changes and other nonhematologic toxic effects occurred in patients who had received more than one course of therapy.

In this trial, the chemotherapeutic regimens did not affect survival. There was little difference in the time to progression of disease among patients who had been previously treated and those who had not. As has been documented in previous ECOG protocols (3), weight loss and

TABLE 1.—Toxicity of phase II drugs

Drug	Treatment status	No. of patients					Nadir time
			Leukopenia (cells/mm^3)		Thrombocytopenia (cells/mm^3)		
		Evaluable	1000-2000	< 1000	40,000-80,000	< 40,000	
ICRF-159	Prior	38	14	0	0	0	7-14 days
	No prior	20	8	4*	0	0	
PZD	Prior	33	5	0	7	5	13-21 days
	No prior	6	2	1	2	1†	
Yoshi-864	Prior	34	0	0	7	1	—
	No prior	5	0	0	1	0	—
CCNU	No prior	21	1	0	0	3†	4-6 wks

* 2 drug deaths.
†1 drug death.

TABLE 2.—Eligibility and treatment responses*

Drug	Treatment status	No. of patients		No. of responses	Site	Median time to progression (wks)
		Entered	Eligible			
ICRF-159	Prior	45	38	0	—	7
	No prior	24	20	1CR	Liver	11
PZD	Prior	41	33	1PR	Lung	7
	No prior	6	6	—	—	9
Yoshi-864	Prior	43	34	1PR	Nodes	6
	No prior	6	5	—	—	18
CCNU	No prior	27	21	2PR	Nodes	15

* The 2 most common causes of ineligibility were lack of histologic confirmation and absence of measurable disease.

prior radiotherapy correlated with increased toxicity. ECOG performance status was associated with improved survival. Patients whose ECOG status was 0 (asymptomatic) (1) survived an average of 34 weeks, while those whose performance status was 1 (symptomatic but ambulatory) lived 21 weeks. Patients who were less than fully ambulatory (ECOG status of 2 or 3) had a median survival of only 11 weeks. Although both CCNU responders suffered severe toxic effects (thrombocytopenia) before showing tumor regression, there was no definite relationship between drug toxicity and either response or survival.

The data presented do not encourage further use of these agents in colorectal cancer. The activity in advanced colorectal cancer previously reported for CCNU (4) could not be confirmed in this study. In this trial, the activity of CCNU was very similar to that previously reported for semustine (1).

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Phase II Trial of α-Lymphoblastoid Interferon Given Weekly as Treatment of Advanced Breast Cancer[1,2]

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Eighteen patients with advanced breast cancer refractory to first-line chemotherapy and hormonal therapy (or estrogen receptor-negative) were treated with human α-lymphoblastoid interferon (Wellferon) in a dose of 30 × 10^6 U/m^2 im weekly. None of 15 patients receiving three or more doses achieved a partial or complete response. Toxicity was substantial and included fatigue, malaise, fever, hematologic suppression, nausea/vomiting, and diarrhea. [Cancer Treat Rep 69:547–549, 1985]

Interferon has demonstrated antineoplastic activity against breast cancer. This has been shown in vitro (against both fresh tumor explants in "clonogenic assay" and passaged cell lines), against mouse mammary tumors, against human breast cancer xenografts in nude mice, and, to some degree, against breast cancer in patients (1,2).

We have studied one form of interferon, human α-lymphoblastoid interferon, in the treatment of patients with metastatic breast cancer. This article reports clinical results of this phase II trial. Biologic studies of interferon's effects on circulating monocytes and lymphocytes were also examined, but will be reported separately.

MATERIALS AND METHODS

Interferon (Wellferon), produced by Burroughs Wellcome Co from a Sendai virus-stimulated Namalwa human B-lymphoblastoid cell line, was provided for study by the National Cancer Institute and the Wellcome Foundation (Kent, England). Wellferon is multispecies α-interferon, approximately 85% pure (1–2 × 10^8 U/mg protein).

Schedule of administration.—Patients received 30 × 10^6 U/m^2 of interferon im once weekly. This schedule is different from that of every 12 hours for 7 days utilized in our previously reported phase I study of interferon (3,4). The weekly schedule was chosen in light of pharmacokinetic data demonstrating prolonged measurable interferon levels (> 100 IU/ml for 1.5–10+ days) following im interferon (4), and in an attempt to give therapy that provided a symptomatic recovery between doses. This schedule had not been previously studied as cancer treatment, although partially purified Cantell α-interferon has been given weekly to some patients with juvenile laryngeal papillomatosis (5). Patients were scheduled to be treated for a minimum of 4 weeks; those who showed disease progression subsequent to that time would be taken off study. Patients who had persistent stable disease would be taken off study after 12 weeks of therapy; those who showed a positive response (minimal, partial, or complete) would continue on therapy until disease progression or 1 year beyond the achievement of a maximal response. Responses were classified by standard criteria as previously defined (3).

Patients.—Eighteen patients were entered on study. All patients were females with measurable and progressing metastatic breast cancer. All patients were either estrogen receptor-negative or had failed first-line hormonal therapy. All patients had failed at least two major chemotherapeutic agents and, at most, one additional chemotherapeutic program beyond regimens

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which included the major chemotherapeutic agents. Patients with disease confined to the chest wall had also failed irradiation therapy. Patients had not received prior interferon therapy and had received no therapy of any sort over the 4 weeks prior to study entry. Patients were excluded from eligibility because of active infection, active brain metastases, or pregnancy. Patients were ambulatory with adequate hematologic status (leukocyte count $\geq 3900/mm^3$, platelet count $\geq 100,000/mm^3$), hepatic status (bilirubin < 1.4 mg/dl, SGOT and SGPT ≤ 100 IU/L), and renal status (creatinine < 2.0 mg/dl). The median age of the patients was 54 years (range, 33-63). Eight patients had been previously tested as estrogen receptor-positive, and five had previously responded positively to systemic therapy. Common sites of metastases were bone (eight patients), lung (six), chest wall (six), lymph nodes (five), pleura (three), liver (five), and subcutaneous tissue other than chest wall (two). Immunologic assessments were preformed prior to and in the course of interferon therapy, but are not the subject of this report.

RESULTS

Efficacy.—No partial or complete responses were achieved. One patient with metastases to lung and chest wall achieved a minimal response after 4 weeks of therapy, followed by disease progression after 12 weeks. Two patients were taken off therapy with stable disease after 12 weeks of treatment. Eight patients had disease progression despite four or more doses of interferon. Six patients tolerated interferon poorly and refused further therapy. Three patients chose to withdraw from therapy after three doses, two after two doses, and one after one dose. The patient who had received only a single dose did not return for evaluation; the other five patients who refused therapy demonstrated disease progression. One additional patient did not refuse interferon but was taken off therapy after three doses of treatment because of rapid disease progression in the brain and lung requiring hospitalization in the intensive care unit. In the 15 patients who received at least three doses of interferon and may be considered evaluable for response, no partial or complete responses occurred.

Toxicity.—Subjective toxicity, primarily malaise and weakness, was substantial and resulted in six patients who refused further therapy as noted above. This generally did not resolve, or resolved only for 1-2 days between doses. Fever and chills occurred universally, with eight patients reaching a peak temperature $\geq 39°C$. Thirteen patients experienced nausea and vomiting, and seven experienced diarrhea. Anorexia was universal, and dysgeusia occurred in one-half of the patients. Eight patients developed mild to moderate leukopenia, but only three developed granulocytopenia. No opportunistic infections occurred. Five patients developed mild to moderate thrombocytopenia. Five patients developed mild transient elevation of SGOT and SGPT. The one patient who received only one dose of interferon developed syncope without evidence of arrhythmia or hypotension shortly after her single dose of interferon.

DISCUSSION

Initial studies of α-interferon as therapy for breast cancer showed promise. Gutterman et al (6) reported six partial responses in 17 patients with breast cancer treated with partially purified Cantell leukocyte interferon. Borden et al (7), reporting an American Cancer Society trial of the same material, noted five partial responders in 23 patients. More recently, however, studies utilizing other forms of α-interferon have been less successful. Although the two studies using Cantell interferon (6,7) had a pooled response rate of 27.5%, this and other recent studies (3,4,8-13) using lymphoblastoid, recombinant α_2, or recombinant α_A interferons have had a pooled response rate of 4% (five responses in 125 patients) (table 1). Considering only the data from the present study (no responses in 15 evaluable patients), one may calculate that there is a $< 5\%$ probability that the true response rate is $> 20\%$. We have tested 11 additional patients with lymphoblastoid interferon in a phase I study utilizing various doses in a different schedule without achieving a partial or complete response (3,4). If one pools the data from our two studies, one may calculate a $< 5\%$ probability that the true response rate is $\geq 11\%$.

If the apparent discordance in results between studies of Cantell α-leukocyte interferon as treatment for breast cancer and studies of lymphoblastoid and other forms of α-interferon is in fact real, it may be due to differences in the materials studied (eg, lymphokines or other products in the Cantell preparation absent from others), to dose/schedule factors, or to patient-related factors. Alternatively, the apparent relative superiority of the Cantell product may be an artifact of a favorable population of small sample size. Further studies with the Cantell product or other partially purified leukocyte interferons may help to resolve this issue. Alpha-lymphoblastoid interferon, as administered in this study to patients with previously treated breast cancer, was ineffective and associated with substantial toxicity. Toxicity failed to meaningfully abate between doses, despite the weekly schedule.

TABLE 1.—Interferon response rate

Reference	α-Interferon	Schedule	No. of responders/ No. evaluable
Gutterman et al (6)	Cantell partially purified leukocyte	3×10^6 U/m^2 or 9×10^6 U im 3 times/wk	6/17
Borden et al (7)	Cantell partially purified leukocyte	3×10^6 U/m^2 im daily × 28	5/23
			Total: 11/40 = 27.5%
Sherwin et al (8)	Recombinant α_A	50×10^6 U/m^2 im 3 times/wk	0/17
Sherwin et al (9)	Recombinant α_A	Variable dose (phase I), $1–136 \times 10^6$ U/m^2 im 3 times/wk	1/9
Silver et al (10)	Lymphoblastoid	2×10^6 U/m^2 or 5×10^6 U/m^2 im daily	1/27
Muss et al (11)	Recombinant α_2	50×10^6 U/m^2 iv daily × 5	0/12
Smedley et al (12)	Recombinant α_A	20×10^6 U/m^2 daily or 50×10^6 U/m^2 im 3 times/wk	2/12
Goodwin et al (13)	Lymphoblastoid	3×10^6 U/m^2 or 0.5×10^6 U/m^2 im 3 times/wk	1/22
Sarna et al (3,4)	Lymphoblastoid	Variable dose (phase I), $0.75–50 \times 10^6$ U/m^2 im twice/day × 7	0/11
Present study	Lymphoblastoid	30×10^6 U/m^2 im every wk	0/15
			Total: 5/125 = 4%

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New Folate Analogs of the 10-Deaza-aminopterin Series: Markedly Increased Antitumor Activity of the 10-Ethyl Analog Compared to the Parent Compound and Methotrexate Against Some Human Tumor Xenografts in Nude Mice[1,2]

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In an extension of our prior studies in murine tumor models, we examined two new folate analogs in the 10-deaza-aminopterin series for antitumor activity against a group of human tumor xenografts in nude mice. In all three xenograft models studied, MX-1 mammary carcinoma, LX-1 lung carcinoma, and CX-1 colon carcinoma, 10-deaza-aminopterin was minimally active, while methotrexate was inactive. In contrast, against the MX-1 and LX-1 tumors, 10-ethyl, 10-deaza-aminopterin at or near the LD_{10} dose (2–4.5 mg/kg) given once per day × 5 produced frank regressions. Activity of this analog against the CX-1 tumor was less, but retardation of tumor growth was observed with some minor regressions. [Cancer Treat Rep 69:551–553, 1985]

Toward the design of new folate analogs with enhanced selectivity of antitumor action, we have sought to exploit (1,2) differences between tumor and normal proliferative tissue at the level of mediated membrane transport and polyglutamylation of these agents. We have found (1–3) that the differential in membrane transport of folate analogs between responsive tumors and normal proliferative tissues of the mouse, which appears to favor greater levels of accumulation of drug in the former, can be accounted for by a difference in specificity for at least one site on the folate molecule, the N^{10} position. Variable specificity for this position among different tissues was also seen (4,5) for polyglutamylation. Since this position is relatively nonspecified (1–3) for inhibition of the intracellular target, dihydrofolate reductase, new analogs modified at this site could have potential for improved therapeutic activity.

In our initial studies of the new N^{10} analog, 10-deaza-aminopterin, we demonstrated (6) antitumor properties superior to methotrexate against a small group of murine ascites tumors. Since this improvement in relative therapeutic activity was predicted by the results (7) of our biochemical and pharmacologic studies with this new analog, additional analogs in this new series were synthesized and subsequently evaluated (4,8,9). These results, again, documented further substantial improvement in antitumor efficacy for some of these analogs, particularly, in the case of 10-ethyl, 10-deaza-aminopterin against metastatic solid murine tumors. Moreover, this improved therapeutic activity also appeared to be explained (4) by a greater differential in both membrane transport inward and polyglutamylation in tumor compared to normal proliferative tissue. In a further extension of this developmental program, we now report the results of limited studies carried out on three human tumor xenografts in nude mice.

MATERIALS AND METHODS

Most of the experimental procedures used during these studies have been described in detail in earlier reports (3,6,9) from our laboratory. All of the folate compounds employed were prepared as the sodium salt in neutral aqueous solution. The synthesis of these compounds has been reported elsewhere (8). Samples of methotrexate used were provided by the Drug Procurement and Synthesis Branch, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD. All compounds employed were analyzed (8) and found to be > 95% pure. For therapy experiments, the human tumors were maintained in nude (Swiss NU/NU) female

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mice by sc transplantation in the axillary region. These tumors were obtained from the Animal and Human Tumor Bank of the Division of Cancer Treatment, National Cancer Institute. Therapy was initiated on Day 2 after transplantation by ip administration of drug on a schedule of once every day × 5. Antitumor effects were expressed as average change in tumor volume (V[mg] ± SE = $4/3\pi r^3$) after therapy. Toxicity was monitored by weight loss and drug-induced deaths.

RESULTS AND DISCUSSION

From data shown in table 1, it can be seen that tolerance of these mice to these analogs varied substantially. Approximate LD_{10} dosages were 1.5, 3, and 4.5 mg/kg for 10-deaza-aminopterin, methotrexate, and 10-ethyl, 10-deaza-aminopterin, respectively. However, in those experiments done with the CX-1 tumor, tolerances of these mice were somewhat less than for the other xenografts. Against the MX-1 tumor (table 1), methotrexate was essentially inactive and 10-deaza-aminopterin was only minimally active, showing slight retardation of growth at tolerated doses. However, the 10-ethyl derivative of 10-deaza-aminopterin showed substantial antitumor activity. At a dose of 2–4.5 mg/kg, reduction in tumor volume was seen (table 1) 1 day after therapy, reaching a nadir 4 days later of 57%–76% reduction. Also, at these doses of 10-ethyl, 10-deaza-aminopterin, a number of animals showed no palpable tumors. Tumor-free mice were observed at 5 days (four of nine mice), 12 days (three of nine), and 26 days (one of nine) after therapy with 4.5 mg/kg.

Against the LX-1 tumor, overall antitumor activity was similar (table 1) to that obtained against the MX-1 tumor. However, treatment with tolerated doses of methotrexate and 10-deaza-aminopterin resulted in some retardation of tumor growth. Again, treatment with 10-ethyl, 10-deaza-aminopterin (2–4.5 mg/kg) resulted in some reduction in tumor volume. At the nadir, 5 days following therapy, the reduction was 20% to 50% of the original tumor. However, unlike the results obtained with the MX-1 tumor, no animals were

TABLE 1.—Antitumor effects of folate analogs against human tumor xenografts

Tumor	Drug	Dose* (mg/kg)	Average tumor volume† (mg ± SE)	Average tumor volume† % change	No. of toxic deaths/total‡
MX-1	—	—	375 ± 42	+468	0/24
	Methotrexate	1.5	334 ± 35	+404	0/12
		3.0	293 ± 31	+346	2/12
		6.0	249 ± 32	+278	7/12
	10-Deaza-aminopterin	0.75	257 ± 24	+289	0/9
		1.5	181 ± 28	+174	1/9
		3.0	87 ± 9	+32	5/9
	10-Ethyl, 10-deaza-aminopterin	1.1	74 ± 11§	+12	0/9
		2.2	31 ± 5§	−57	0/9
		4.5	16 ± 4§	−76	2/9
LX-1	—	—	587 ± 62	+355	0/18
	Methotrexate	1.5	435 ± 39	+238	0/6
		3.0	299 ± 32	+132	1/9
		6.0	247 ± 38	+92	3/9
	10-Deaza-aminopterin	0.75	317 ± 29	+145	0/6
		1.5	264 ± 33	+105	0/9
		3.0	151 ± 25	+25	2/9
	10-Ethyl, 10-deaza-aminopterin	1.1	231 + 22	+79	0/6
		2.2	103 ± 16	−20	0/9
		4.5	71 ± 18	−47	1/9
CX-1	—	—	263 ± 33	+143	0/15
	Methotrexate	1.5	282 ± 31	+162	0/6
		3.0	256 ± 27	+138	2/9
		6.0	78 ± 13	−28	7/9
	10-Deaza-aminopterin	1.5	259 ± 38	+139	0/9
		3.0	188 ± 25	+74	4/9
	10-Ethyl, 10-deaza-aminopterin	2.0	149 ± 24	+38	0/11
		3.0	87 ± 16	−19	1/10
		4.5	76 ± 14	−30	5/13

*1 dose per day × 5 beginning on Day 2 (3 experiments of 3–6 mice/group).

†Tumor volume ± SE of the mean on the 5th day after cessation of therapy. Tumor volume at the start of therapy was 66 ± 7 mg (MX-1), 129 ± 11 mg (LX-1), and 108 ± 12 mg (CX-1).

‡Animals showed frank weight loss at the time of death (period of observation was 21 days).

§1/9 (1.1 mg/kg), 2/9 (2.2 mg/kg), and 4/9 (4.5 mg/kg) treated mice were tumor-free.

found to be tumor-free following therapy with this analog.

Treatment of the CX-1 tumor with tolerated doses of methotrexate or 10-deaza-aminopterin was essentially without effect. At tolerated doses of 10-ethyl, 10-deaza-aminopterin (3 mg/kg), tumor growth appeared to be completely inhibited within the period during and shortly after therapy with some indication of regression. However, this was difficult to quantitate in view of the slow rate of growth of this tumor. In any event, the effect of this agent was substantially less than that obtained against the two other xenografts.

These results with human tumor xenografts extend our earlier (6,9) studies with model murine tumor systems. They provide further documentation for the markedly superior antitumor properties of 10-ethyl, 10-deaza-aminopterin in comparison to its parent derivative and methotrexate.

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Treatment of Small Cell Lung Cancer With 6-Mercaptopurine: A Phase II Study[1]

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Although about 80% of the patients with small cell lung cancer (SCC) achieve regression during chemotherapy, the duration of response is only 10–12 months. The need for more active agents is still urgent, and as the literature does not reveal an adequate testing of 6-mercaptopurine (6-MP) in SCC (1–3), this study was designed to evaluate the activity of 6-MP in this disease entity.

PATIENTS AND METHODS

All patients entering the study had histologically verified SCC and had received prior chemotherapy with at least four of the following drugs: lomustine, vincristine, cyclophosphamide, methotrexate, doxorubicin, etoposide, teniposide, cisplatin, hexamethylmelamine, or vindesine. No chemotherapy had been given for 4 weeks (6 weeks for lomustine) before the start of 6-MP. Before entering the study each patient had a complete physical examination, chest x-ray, and laboratory tests including a cbc, serum creatinine, bilirubin, alkaline phosphatase, and SGOT.

Cbcs were repeated every week; the other tests were repeated every 3 weeks. A wbc count $> 4.0 \times 10^9$/L and a platelet count $> 100 \times 10^9$/L were required for inclusion in the study unless lower counts were related to bone marrow carcinomatosis. All patients had measurable or evaluable disease, and response was defined according to World Health Organization recommendations (4). To be evaluable for response the patients should have received at least two courses of 6-MP. 6-MP was given in a dose of 250 mg/m^2 orally daily for 5 days every 3 weeks. Each daily dose was divided in two.

RESULTS

Thirty-six patients entered this study (table 1); 10 died within 2 weeks with disease progression, and they are excluded from further analysis. Fifteen patients were evaluable for response. The median number of courses was three (range, two to five). No response was observed in this group of patients.

TABLE 1.—Patient characteristics and toxicity

Characteristics	No. of patients
Total	36
Sex	
Male	24
Female	12
Median age in yrs (range): 60 (42–76)	
Median performance status (range)*: 2 (1–3)	
Evaluable patients	
Toxicity and response	15
Toxicity only	11
Hematologic toxicity ($\times 10^9$/L)	
Median wbc count (range): 2.8 (1.1–6.8)	
Median platelet count (range): 68 (13–268)	
Hepatic toxicity	7
Gastrointestinal toxicity	11

* Eastern Cooperative Oncology Group criteria.

DISCUSSION

In the present study 6-MP failed to demonstrate any activity in patients with SCC previously treated with combination chemotherapy. It is concluded that 6-MP deserves no major role in the future treatment of SCC.

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Phase II Study of Amsacrine in Gastric Carcinoma: A Southwest Oncology Group Study[1,2]

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Amsacrine (AMSA) is an acridine derivative with limited activity in gastrointestinal malignancies (1-4). This Southwest Oncology Group study evaluated its effectiveness and toxicity in patients with advanced gastric cancer.

MATERIALS AND METHODS

Forty-five patients with measurable advanced gastric cancer were treated with AMSA on SWOG 7931 and SWOG 7841. All patients had a life expectancy > 6 weeks, performance status of 0-3, wbc count > 4000 mm/^{3}, platelet count > 100,000/mm^3, and bilirubin < 5 mg/dl. The good-risk patients were < 65 years of age and had had no prior irradiation; creatinine clearances were > 20 ml/minute and bilirubin was < 2 mg/dl. They received 120 mg/m^2 of AMSA in 500 ml of 5% dextrose in water infused over 1 hour. Poor-risk patients received 90 mg/m^2. The treatment was repeated in 21 days or after adequate marrow recovery. In the absence of significant myelosuppression, the dosage was increased by 30 mg/m^2 in subsequent courses.

Patients were evaluable after one course of therapy. A partial response was defined as a > 50% decrease in the size of all measured lesions, sustained for 4 weeks.

RESULTS

The patient characteristics, treatment results, and hematologic toxic effects in this study are summarized in table 1. Forty-two of the 45 treated patients were evaluable for response. No complete responses were observed in either treatment group. One patient with liver metastases on radionuclide scanning achieved a partial remission after two courses of treatment. However, he experienced progressive weight loss and died 3 weeks after his fourth treatment. This patient had received no prior therapy. Fourteen other patients had disease stabilization.

TABLE 1.—Patient characteristics and response*

Characteristics	Good-risk	Poor-risk
Treated	24	21
Median age in yrs (range)	58 (41-64)	70 (45-82)
Evaluable	23	19
Prior chemotherapy	7	9
Performance status 0-1	16	8
Performance status 2-3	7	11
Dose escalation	6	8
Hematologic toxicity (wbc count, cells/mm^3)		
< 1000	1	0
1000-1999	3	2
2000-2999	10	9
Response		
Partial response	1	0
Stable disease	8	6
Disease progression	14	13

* Unless otherwise specified, values = No. of patients.

Minimal hematologic toxicity was experienced. One patient developed life-threatening leukopenia after receiving 120 mg/m^2 of AMSA, with a nadir wbc count of 500/mm^3 during her third course. Five other patients had severe leukopenia. No cardiac arrhythmias or severe gastrointestinal or neurologic toxic effects were reported.

DISCUSSION

As noted in previous studies (2,3), the administration of AMSA at 3-week intervals had no significant activity in patients with advanced gastric cancer.

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Phase II Study of 4-Demethoxydaunorubicin in Advanced Ovarian Carcinoma[1,2]

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4-Demethoxydaunorubicin (4-DMDR) is an anthracycline analogue that differs from daunorubicin (DNR) by the replacement of the methoxy group in position 4 with a proton.

When given iv, 4-DMDR is five times more potent than DNR against L1210 and P388 ascitic leukemia (1-3). It is also more active against Gross leukemia than either DNR or doxorubicin (DOX). Unlike DNR or DOX, 4-DMDR has clear biologic activity when administered orally (4,5). In view of these favorable characteristics, and because 4-DMDR is significantly less cardiotoxic than either DNR or DOX in mice, dogs, and rabbits (3,4), the drug was introduced into clinical trials.

MATERIALS AND METHODS

Patients

Patients with recurrent ovarian carcinoma were eligible for this study. Eligibility requirements included bidimensionally measurable disease, a performance status of $\geq 50\%$ on the Karnofsky scale, no clinical or laboratory evidence of cardiac dysfunction, serum bilirubin < 1.5 mg/dl, and a serum creatinine < 2.5 mg/dl.

There were 18 patients with adenocarcinoma and one patient with a granulosa cell tumor. All patients had received standard chemotherapy for ovarian carcinoma, usually cyclophosphamide, cisplatin, and doxorubicin or vinblastine. Sixteen patients had received prior doxorubicin, with a median cumulative dose of 375 mg/m^2 (range, 240-470). Four patients had received pelvic radiation therapy.

Treatment

In patients considered to have normal bone marrow reserve, 4-DMDR was given at a dose of 12.5 mg/m^2 iv every 3 weeks. In marrow-impaired patients, the dose was reduced to 10 mg/m^2. Eighteen of 19 patients received at least two courses of 4-DMDR.

RESULTS

Among the 19 patients treated, 18 are evaluable for toxicity and 17 are evaluable for response. One patient was lost to follow-up after one treatment and was not evaluable for toxicity or response. A second patient whose measurable disease criteria were not confirmed was evaluated for toxicity only. No therapeutic responses were observed among the 17 evaluable patients.

The toxicity of 4-DMDR was mild, with myelosuppression being the most common toxic effect (table 1). Nausea and vomiting of a mild degree occurred in 11 patients; mucositis was seen in only one patient. In the few patients who could be evaluated for alopecia, no significant hair loss was observed. Most patients had pre-existing alopecia. No clinical evidence of cardiac dysfunction was observed in any of the patients.

DISCUSSION

Doxorubicin is an effective drug in the treatment of ovarian carcinoma (6); however, the extended administration of this anthracycline is limited by dose-related cardiotoxicity. 4-DMDR is a new anthracycline analogue with much less cardiac toxicity in animal model systems. 4-DMDR also has demonstrable therapeutic activity in previously treated leukemic patients (7). However, in the group of heavily pretreated patients with ovarian cancer presented here, we have seen no antitumor activity. We conclude that at the route, dose, and schedule used, this drug is inactive in heavily pretreated patients with ovarian cancer.

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TABLE 1.—Patient characteristics and drug toxicity

Characteristics	No. of patients
Mean age in yrs (range): 55 (25–69)	
Mean performance status (range): 70% (50%–90%)	
Stage of disease	
III	13
IV	6
Sites of disease	
Pelvis	18
Abdomen	10
Liver	7
Lung	1
Spleen	1
Prior radiation therapy	5
Prior doxorubicin (mg/m^2)	15
Median: 375	
Mean: 360	
Range: 240–470	
Hematologic toxicity	
Myelosuppression (nadir)	
Wbc count (× 10^3 cells/mm^3)	
Median: 3.0	
Mean: 2.7	
Range: 0.3–6.1	
Hgb (g/100 ml)	
Median: 9.2	
Mean: 9.2	
Range: 7.3–10.8	
Platelet count (× 10^3 cells/mm^3)	
Median: 129	
Mean: 141	
Range: 18–395	
Nonhematologic toxicity	
Nausea and vomiting	11
Mucositis	1
Liver dysfunction	1

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Localized Subcutaneous Toxicity Following Bleomycin Administration

Localized tissue toxicity after infiltration of an iv antitumor agent is well documented with drugs such as doxorubicin, daunorubicin, and vincristine (1,2). Lesions caused by these agents begin as tender, indurated erythematous areas and frequently progress to severe skin ulcerations; such complications add to the morbidity caused by chemotherapy treatments. We report a very unusual reaction of this kind to a sc injection of bleomycin.

CASE REPORT

A 61-year-old obese woman with a 2-year history of lymphocytic lymphoma in leukemic phase was admitted to Montefiore Medical Center in October 1983 for treatment of progressive disease. A treatment regimen of vinblastine, bleomycin, lomustine, and prednisone was instituted after discontinuance of CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) because of cardiotoxicity and progression of disease after C-MOPP (cyclophosphamide, methotrexate, vincristine, prednisone, and procarbazine). As a test dose, 1 IU of bleomycin was administered sc the day prior to the patient's first course of chemotherapy without any adverse reactions. She was then given 9 IU of bleomycin sc in her right upper arm along with the other agents. The patient complained only of minimal burning, but a hematoma and ecchymosis developed over the next 2 days.

On Day 13 of this chemotherapy course, she was seen as an outpatient for further chemotherapy. The hematoma and ecchymosis were still present at this time. Two subsequent courses of bleomycin were administered iv. At Day 48 of the chemotherapy course, the initial injection site lesion was noted to have increased in size to a 2 × 3-cm area of induration with a central ulcer measuring 0.5 × 1 cm. Although tender, the lesion did not restrict arm movement.

A punch biopsy of the lesion lateral to the ulceration consisted of full-thickness skin and a portion of subcutaneous fat. The epidermis showed hyperkeratosis and slight acanthosis. Scattered individually necrotic keratinocytes were present. The dermis was sclerotic with obliteration of the papillary reticular dermal interface and atrophy of skin appendages. There was irregular thickening and horizontal deposition of the dermal collagen bundles. Many of the fibroblasts showed abundant cytoplasm, a stellate configuration, and prominent nucleoli. Elastic tissue stain showed focal fragmentation but preservation of elastic tissue. Telangiectasia with prominent endothelial lining cells was present in the upper third of the dermis. Dense sclerosis extended to the subcutaneous septae and the interstitium of the adipose tissue. A medium-sized subcutaneous arteriole at the lower border of the biopsy showed fibrinoid necrosis and thrombosis. There was no significant inflammatory infiltrate in any portion of the biopsy. Except for the preservation of elastic tissue, the changes most closely resembled late radiation dermatitis.

The patient received no further chemotherapy. She had a downhill course and died of progressive lymphoma. Twelve weeks after the bleomycin injection, the lesion had progressed to a 3 × 3.5-cm area of induration, and a 1 × 2-cm area of central ulceration. Permission for a post mortem examination was denied.

DISCUSSION

Bleomycin is one of a family of antibiotic peptides isolated from the fungus *Streptomyces verticullus*. Its major toxicity is pulmonary, causing a subacute or chronic pneumonitis that progresses to interstitial fibrosis (3). The most frequent side effects of bleomycin involve the integument and mucus membranes. These consist of erythema, rash, striae, vesiculation, hyperpigmentation, and tenderness of skin. Hyperkeratosis, nail changes, alopecia, pruritus, and stomatitis have also been reported.

Seyfer and Solimando (4) have reported bleomycin skin toxic effects localized to hands. In their cases, the clinical picture ranged from severe tenderness with or without blistering to severe swelling of the digital pulps that progressed to open cracks, exposing the subjacent fat. These lesions were unrelated to the areas of bleomycin injections.

Pandeya et al (5) report an unusual localized toxicity in a patient with recurrent metastatic melanoma who received 15 IU of bleomycin im twice weekly to a total dose of 225 IU, and 100 mg of lomustine. Within 3–4 months after the start of her therapy, areas of subcutaneous infiltration and necrosis were noted at the injection sites. A 15 × 20-cm necrotic area was excised and subsequently skin grafted. Pathologic examination of the excised tissue was not reported and it is unclear from the report how many injections had been given.

Our case represents an unusual local cutaneous reaction to a sc injection of bleomycin that, to our knowledge,

has not been previously reported. Clinically, it resembled the well-described infiltration of an iv agent such as doxorubicin in that a clearly demarcated wound progressed to form a central eschar (1). However, our patient's lesions progressed far more slowly than lesions typically seen following a doxorubicin extravasation. Although an ecchymosis was noted within 2 days, it was 7 weeks before the lesion ulcerated. In addition, the lesion was histopathologically similar to radiation dermatitis, despite the absence of any radiation therapy. It is interesting to note that no other signs of bleomycin toxicity developed and that the lesion did not flare after additional treatment with iv bleomycin. Unfortunately, the woman died prior to the resolution of the lesion and before further determinations of the pathological features and the clinical course could be made.

We report here an ulcerated lesion believed to be secondary to a sc injection of bleomycin. Although mucocutaneous toxic effects have been well documented following the administration of bleomycin, such incidents are the result of the systemic effect of the drug. Local tissue toxicity secondary to a bleomycin injection has been reported as necrosis only once (5), and a localized ulceration with sclerosis as reported here is also an extremely unusual reaction to bleomycin, and, to our knowledge, has not been previously reported.

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Immediate Hypersensitivity to Mannitol: A Potential Cause of Apparent Hypersensitivity to Cisplatin

Immediate hypersensitivity reactions attributed to cisplatin are well known, occurring with an incidence of 1%-5% in series of patients where the drug is used alone, and up to 20% when used in combination with other cancer chemotherapeutic agents (1-4). Both severe (hypertension, bronchospasm) and mild (rash, urticaria, paresthesia) reactions have been reported frequently. However, hypersensitivity reactions to mannitol (1-5) have been reported frequently, and have all been severe (5). Here we cite a case of immediate hypersensitivity to mannitol, and emphasize how important it is to recognize this toxicity in patients receiving such anticancer drugs as cisplatin.

A 28-year-old male production engineer with a past history of childhood asthma and allergic rhinitis was admitted to the hospital after a 1-month history of insidious onset of dyspnea and mild pleuritic chest pain followed by collapse. The patient had clinical and lung scan findings consistent with multiple pulmonary emboli, but, in addition, a large abdominal mass and a small right testicular mass were noted. Following appropriate treatment for and recovery from his pulmonary emboli, he underwent laparotomy, at which a large retroperitoneal mass of tumor was biopsied. Histology showed necrotic nonseminomatous germ cell tumor. Alpha-fetoprotein level after surgery was 154 μg/L (normal range, 0-12). Three courses of chemotherapy with cisplatin, vinblastine, and bleomycin (PVB) at 4-week intervals were planned. Two liters of dextrose-saline was given as hydration to be followed by 400 ml of 10% mannitol over 30 minutes to enhance diuresis prior to cisplatin. However, after receiving 150 ml of mannitol, the patient had a sudden onset of dyspnea, generalized tight pain in the chest, and a pruritic rash. Examination revealed erythematous wheals over his trunk and arms, pulse 80 beats per minute and regular, blood pressure 110/80 mm Hg, and clinical signs of bronchospasm. Mannitol was discontinued and iv promethazine (25 mg) was given. The reaction resolved within 15 minutes. The formulation of cisplatin includes mannitol, so after a 3-day interval, chemotherapy was restarted with a special preparation of mannitol-free cisplatin supplied by the manufacturer as a powder and prepared by reconstitution to a concentration of 1 mg/ml with sterile water. It was then administered as a 1-hour infusion in 500 ml *n*-saline, without pretreatment with mannitol. No hypersensitivity occurred.

The patient was given two further courses of PVB over a 3-month period using mannitol-free cisplatin without untoward effects. During this period, he required inferior

venacaval ligation for control of further pulmonary emboli and a right orchidectomy which revealed differentiated teratoma. Serum alpha-fetoprotein levels fell to within normal limits. Following the third course of PVB, the patient underwent retroperitoneal lymph node dissection; histology of the specimen showed a large amount of necrotic debris and fibrosis and a small area of immature teratoma. Since that time, he has had a left orchidectomy and treatment with depot testosterone, but has remained well for 36 months with negative markers and a normal computerized tomographic scan and chest x-ray. No sensitivity skin testing was performed.

This case clearly demonstrates an immediate hypersensitivity reaction to mannitol in a patient with an atopic history. Since cisplatin is supplied as a lyophilized powder containing 10 mg of cisplatin and 100 mg of mannitol in a 10-ml vial, and additional mannitol is frequently given before cisplatin, it is possible that at least some of the reported instances of hypersensitivity to cisplatin in patients receiving this drug for cancer chemotherapy have really been hypersensitivity to mannitol. Khan et al (4) clearly demonstrated immediate hypersensitivity to cisplatin alone in their patient using skin tests, and in other reports, hypersensitivity due to cisplatin was assumed because of close temporal relationship and uncomplicated pretreatment with mannitol. However, there are multiple previous reports of patients where hypersensitivity could have been due to mannitol rather than cisplatin (1-3). It is important to exclude hypersensitivity to mannitol in cases such as these so that patients are not denied a drug which is so beneficial in their management. Since mannitol is added to cisplatin powder merely as a bulking agent to facilitate dissolution, it is possible to obtain and administer cisplatin without mannitol as we did in our patient.

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Hyperphosphatemic Renal Failure Following Treatment of Acute Nonlymphatic Leukemia

Acute renal failure secondary to massive tumor lysis and consequent hyperphosphatemia has been reported following treatment of acute and chronic lymphoproliferative disorders (1). We have recently cared for a patient in whom this syndrome occurred following the treatment of acute nonlymphatic (myelogenous) leukemia (ANLL).

The patient was a 72-year-old white Italian-American retired laborer. In 1971, a diagnosis of polycythemia vera was made after he presented with pruritus and erythrocytosis. In the next 12 years, he was treated sequentially with phlebotomy, ^{32}P (total dose, 6.5 mCi), chlorambucil, and busulfan. He was well, with borderline elevated blood cell counts, until June 1983. Then, at routine bimonthly evaluation, he was found to be anemic. All medications were discontinued, but his anemia progressed mildly, and leukocytosis and thrombocytopenia developed. By January 1984, the following laboratory data were present: Hgb of 8 g/dl; hematocrit of 25%, with a mean corpuscular volume of 102 and a mean corpuscular hemoglobin of 34; wbc count of 30,000/mm^3; and platelet count of 90,000/mm^3. A chromosome karyotype (peripheral blood with G-banding, via Dr. R. Schmidt, MetPath Laboratories) showed 47 XY 8+ (trisomy 8), and a bone marrow aspirate showed marked leukocytosis with a left shift but without blastic transformation. The patient was considered to have a myelodysplastic syndrome (refractory anemia). In April 1984, his wbc count rose precipitously to > 200,000/mm^3. A diagnosis of ANLL (French-American-British classification of 1) was made and he was admitted to the Albert Einstein College Hospital. He was started on allopurinol, iv fluids to ensure urine output at 2 L/day, and hydroxyurea (4500 mg daily). Within 7 days, his wbc count had fallen to 100,000/mm^3. On the ninth hospital day, he became oliguric (urine output 200 ml/day), with a phosphate of 22 mg/dl (table 1). Despite immediate treatment with hypertonic glucose and insulin (for hyperkalemia), peritoneal dialysis (for hyperphosphatemia), transfusions of packed rbcs and platelets, and vigorous supportive care, his condition steadily worsened, with features suggestive of the adult respiratory distress syndrome. He died on the 19th hospital day.

TABLE 1.—Patient data

	Hospital Day										
	1	3	5	7	8	9	11	13	15	17	19
Hgb (g/dl)	7.1	7.7	6.6	7.1	6.9	7.1	7.1	9.2	9.6	9.6	8.1
Wbc ($\times 10^3/mm^3$)	203	126	138	100	61.2	37	7.0	2.7	0.8	0.4	0.4
BUN (mg/dl)	16	27	25	29	39	111	107	82	93	87	83
Creatinine (mg/dl)	1.2	2.2	1.7	2.0	3.0	7.0	6.4	5.3	6.8	4.5	4.6
Potassium (mEq/L)	4.8	4.3	4.3	3.9	3.7	8.0	5.2	4.5	5.3	4.4	5.3
Uric acid (mg/dl)	11.5	9.1	6.9	7.1	8.2	9.6	7.8	14.5	14.1	11.3	—
Calcium (mg/dl)	8.2	7.4	7.8	8.1	7.2	6.4	7.0	7.4	6.8	7.0	—
Phosphate (mg/dl)	2.3	3.8	2.3	3.0	7.7	22	22.4	16.8	11.7	8.1	—
Urine											
pH	5		6			6					
Creatinine (mg/total vol)						36	18				
Uric acid (mg/total vol)						35	29				
Calcium (mg/total vol)							4.7				
Phosphate (mg/total vol)							21				
Doxorubicin											
Cytarabine											
Hydroxyurea →											
Allopurinol →											
Dialysis →											

The syndrome of hyperphosphatemic renal failure has not, to our knowledge, been described in ANLL. The rapid destruction of cells, under the influence of cytotoxic chemotherapy, allows for the massive release of phosphate. With hyperphosphatemia, calcium salts precipitate in the renal tubules and calyces, leading to nephrocalcinosis and renal failure. The Ca × P solubility product in this patient was markedly elevated above the limits at which nephrocalcinosis occurs (2).

Allopurinol may also have contributed to renal failure. A hypoxanthine analog, allopurinol is a potent inhibitor of xanthine oxidase. Its use results in systemic accumulation of nonurate purines, xanthine and hypoxanthine, and may predispose to the precipitation of xanthine stones. Although these nonurate purines are more soluble in serum than is uric acid, they are less soluble in urine. In addition, alkalinization of the urine, which enhances uric acid solubility greater than tenfold, only increases xanthine solubility twofold. Nonetheless, xanthine-induced acute renal failure associated with chemotherapy is very rare, and as hyperphosphatemic-induced renal failure, has only been reported in acute and chronic lymphoproliferative disorders (3).

In spite of recommended procedures to avoid renal failure (the institution of allopurinol therapy and vigorous hydration), uremia developed in our patient. Two additional prophylactic maneuvers have been suggested; neither is without risk. The first is pretreatment with aluminum hydroxide to induce phosphate depletion. Experimentally, phosphate-depleting regimens of short duration lower serum phosphate minimally and markedly impair the renal capacity to excrete an acute phosphate load (4). Thus, the approach may exacerbate rather than alleviate hyperphosphatemia following chemotherapy. The second is alkalinization of the urine. Urine alkalinization to prevent precipitation of urate and xanthine may actually enhance tubular deposition of phosphate, and thus should be considered with caution.

Perhaps the best approach would be to institute leukapheresis prior to therapy to reduce the tumor burden in patients with marked leukocytosis, to obviate massive tumor lysis and hyperphosphatemia.

In the patient described, once renal failure occurred, peritoneal dialysis was instituted. Either peritoneal or hemodialysis may be used in this clinical situation, since both effectively clear phosphate and xanthine. In spite of dialysis and normalization of serum electrolytes, the patient remained oliguric and eventually succumbed to his disease.

In addition to this unusual complication of therapy, the patient presents the fascinating progression from polycythemia vera to ANLL via a myelodysplastic syndrome. His karyotype (trisomy 8) has been reported to occur in patients exposed to noxious chemicals (5) and may reflect the leukemogenic influence of his treatment for polycythemia.

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October 17, 1984

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Effect of Cyclophosphamide on the Mitomycin-Induced Syndrome of Thrombotic Thrombocytopenic Purpura[1]

We report here our findings and management of a patient with the syndrome of thrombotic thrombocytopenic purpura. We have recently studied the cellular and humoral immunity of a patient with breast carcinoma who developed this syndrome on a mitomycin-containing chemotherapy regimen. We found overreactivity of the cytotoxic suppressor T cells. Cyclophosphamide exerted a regulatory effect on these suppressor cells and temporarily reversed hematologic and renal dysfunction after treatments with steroids and plasmapheresis had failed to do so.

CASE REPORT

A 36-year-old woman presented with a 4-year history of a hormone-independent breast carcinoma that had recurred at the sites of the ipsilateral chest wall and pleura 27 months after the primary surgical treatment. The disease was refractory to treatments with CMF (cyclophosphamide, methotrexate, and 5-FU), and doxorubicin with vincristine. In February 1982, a regimen combining mitomycin and vinblastine was started, and the treatment resulted in good antitumor effect. In September 1982, after seven courses (47 mg/m^2 of mitomycin), the patient presented with petechiae, ecchymoses, and epistaxis. The platelet count was 31,000/mm^3, the hematocrit was 26.6%, and schistocytes were present in the peripheral blood smear. Bone marrow was normocellular, with a normal differential count and normal morphology of the nucleated blood cells. No tumor cells were present. Creatinine clearance was 55 ml/minute. The syndrome of thrombotic thrombocytopenic purpura with microangiopathic hemolytic anemia and renal impairment was diagnosed. Treatments with high-dose corticosteroids and plasmapheresis remained without effect.

Because breast tumor growth reactivated, the patient was referred to this institution for further management in January 1983. She appeared in good general health and was fully ambulatory. Tumor was present in the right anterior chest wall, in the form of an erythematous rash, and in the right pleura. There were petechiae and ecchymoses over the lower extremities, but the spleen was not enlarged. The hematocrit was 28%, the platelet count was 35,000/mm^3, and the wbc count was 4800/mm^3 with 49% lymphocytes. Bone marrow was unremarkable. Megakaryocytes were normal in number and in appearance. Fibrinogen degradation products were elevated, but prothrombin time was normal. Protein electrophoresis was normal. Skin test reactivity was hypoergic, with weak reactions to four antigens and no response to two. The lymphocyte count was 2400/mm^3, and 81.9% were T cells. The helper:suppressor T-cell ratio was 0.53 (table 1).

Because of these immunoregulatory abnormalities, treatment was started with cyclophosphamide at 100 mg/m^2 daily orally. The hematologic improvement was immediate. Petechiae and ecchymoses resolved, and the platelet count fluctuated between 70,000/mm^3 and 110,000/mm^3. The creatinine gradually returned to 1.0 mg/dl. After 3 weeks of continual treatment with cyclophosphamide, the immunological parameters also reversed toward normal (table 1). Tumor growth arrested, and the patient went home on this treatment. Two weeks later, treatment-induced pancytopenia occurred. It did not resolve with a dose reduction of cyclophosphamide to 60 mg/m^2. The patient returned to the hospital at the end of March with recurrent thrombotic thrombocytopenic purpura and tumor progression. The platelet count was 24,000/mm^3, and the hematocrit was 33.3%. Tumor cells were now also present in the bone marrow. Creatinine was 1.4 mg/dl, and large amounts of protein, hemoglobin, hemosiderin, and granular casts were contained in the urine sediment. The ratio of helper:suppressor cells was 0.48. An attempt to recapture control of tumor and immune response by treatment with pulse-dose cyclophosphamide failed, and the patient died 2 weeks later from thrombocytopenic pulmonary hemorrhage.

[1]Supported by an allotment from the UCF Annual Giving.

TABLE 1.—Time course of immune status in relation to treatment

Date (mo/day/yr)	Treatment	Platelet count ($\times 10^3/\mu l$)	Creatinine (mg/dl)	% cells with E-rosette receptors*	Ratio of helper: suppressor T cells†	Tumor status
1/25/83	None	35	1.6	81.9	0.52	Progressive
2/9/83	1 wk of cyclophosphamide‡	100	1.4	94.3	0.57	Stable
2/22/83	3 wks of cyclophosphamide‡	63	1.0	60.0	1.04	Stable
3/22/83	None (~2 wks off cyclophosphamide)	27	1.4	95.8	0.48	Progressive

* Normal range = 72.1% ± 13.5%.
† Monoclonal antibodies were used to identify the lymphocyte subsets.
‡ 100 mg/m^2 orally every day.

DISCUSSION

The syndrome of thrombotic thrombocytopenic purpura has only recently become recognized in patients with carcinoma (1). It consists of microangiopathic hemolytic anemia, thrombocytopenia, and renal failure. It is associated with certain tumor types (primarily gastric cancer). Typically, tumors are of minimal size, or not at all clinically evident, and they are well controlled with mitomycin-containing regimens. The pathogenesis is unknown, but defects in the humoral immunity and predisposing genetic factors have been implicated (2,3).

Plasmapheresis improved the hematologic function in three cancer patients, but had no effect on the renal function (2). Furthermore, antibodies removed from the immune complexes of one patient did not react with mitomycin.

Because of its familial occurrence in otherwise healthy individuals, genetic factors have been implicated (3). Interestingly, both patients who had their major histocompatibility genes identified expressed markers of the Cw3 gene. Patients with systemic lupus erythematosus (SLE) frequently carry this alloantigen (4), and Smolen et al (5) recently found that a reversed helper:suppressor T-cell ratio identified those patients with SLE whose disease manifested with thrombocytopenia and renal dysfunction.

Genetic susceptibility for the development of mitomycin-induced thrombotic thrombocytopenic purpura may explain its predilection for certain tumor types and its preferential occurrence during the controlled disease stages. Since improvement of hematologic and renal function occurred in our patient with treatment directed at the destruction of cytotoxic suppressor T cells, dysregulation of cell-mediated immunity may play a role in the pathogenesis of this syndrome. Treatment with cyclophosphamide at a dose that suppresses the overreactive suppressor cells, but not the hematopoietic cells, may therefore provide a rational treatment approach.

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[3]We thank Dr. James Ruben for critical evaluation of the immune test results and Miss Lori Shepard for typing the manuscript.

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Rational Therapeutics: A Seminar in Oncology

Anaheim, California, June 1–2, 1985

This Tenth Annual Symposium, offered by the Department of Pharmacy Service, City of Hope National Medical Center (Duarte, CA), is designed to provide members of the health care team with current and clinically useful information in the management of the cancer patient. The program will feature topics on antimicrobial strategy in febrile cancer patients, cancer pain management, ip chemotherapy, prediction of response to chemotherapy, gynecologic malignancies, "oncology: 1985," and "pearls and perils in oncology." In addition, several clinical tutorials covering specific areas will be available.

The meeting will be held at the Disneyland Hotel; the cost is $120. Twelve hours of accreditation from the California State Board of Pharmacy and California Board of Registered Nursing have been approved. Physicians attending the conference may report up to 12 hours of formal category I credit towards the California Medical Association Certificate of Continuing Medical Education.

For further information, contact: Dwight Kloth, PharmD, Program Chairman, Department of Pharmacy Services, City of Hope National Medical Center, 1500 E Duarte Rd, Duarte, CA 91010.

Cancer Progress

New York, New York, June 10–11, 1985

This 2-day national conference will include such topics as: interferon, interleukins and lymphokines, oncogenes, HTLVs, liposomes, monoclonal antibodies, DNA probes, biotechnology in cancer diagnosis and treatment, pain control, advances in medical imaging, drug delivery, latest developments in instrumentation, devices, prevention, and changing perspectives for treatment.

The meetings will be held at the New York Hilton.

For further information, contact: Sharon Curry, Communitech, P.O. Box 67, Yorktown Heights, NY 10598 (telephone: 914-245-7764).

Critical Care and Medical Management of the Cancer Patient

Buffalo, New York, June 20, 1985

Roswell Park Memorial Institute is conducting monthly continuing education programs in oncology for community practitioners, to disseminate information on the newest methods in the diagnosis and treatment of cancer. Each monthly seminar is organized by a different department of the Institute and covers a different clinical specialty. The seminars qualify for Category I credit toward AMA Physician's Recognition Award, and are submitted for approval for credit by the American Academy of Family Physicians and Category 2-D credit by the American Osteopathic Association. The seminars are sponsored by the Education and Cancer Control Program of the Institute and by the American Cancer Society, New York State Division and Erie County Unit.

The topic of the June seminar is critical care and medical management of the cancer patient (chairperson: Dr. Ramachandran Nambisan). The fee is $45 for physicians or $15 for residents and allied health professionals; there is no fee for students. Preregistration is encouraged.

For further information, contact: Gayle Bersani, RN, Coordinator of Continuing Education Programs, Education Department, Roswell Park Memorial Institute, 666 Elm St, Buffalo, NY 14263 (telephone: 716-845-2339).

Fourth International Conference of Environmental Mutagens

Stockholm, Sweden, June 24–28, 1985

Satellite symposia related to this meeting are offered on the following topics:
Genetic Toxicology of the Diet (Copenhagen, Denmark, June 19–22);

Risk and Reason: Risk Assessment in Relation to Mutagens and Carcinogens (Oslo, Norway, June 20–22);

Monitoring of Occupational Exposure to Genotoxicants (Helsinki, Finland, June 30–July 2, 1985).

For further information, contact: Congress Office, ICEM-85, Stockholm Convention Bureau, Box 1617, S-11186 Stockholm, Sweden.

Fifth Annual Epidemiology Summer Program

Amherst, Massachusetts, July 28–August 16, 1985

The University of Massachusetts will host a 3-week summer program in epidemiology which will include both methodologic and substantive courses and will offer classes for participants seeking an introduction to modern epidemiologic concepts as well as those desiring a review of recent developments in epidemiologic thinking. Thirteen courses will be offered; invited faculty include excellent teachers and prominent researchers from leading universities. Registrants may receive credits toward graduate degrees, continuing medical education, or certification maintenance in industrial hygiene.

For further information, contact: New England Epidemiology Institute, Department SC-4, P.O. Box 57, Chestnut Hill, MA 02167 (telephone: 617-734-9100).

Joint Conference of the 17th International Leukocyte Culture Conference and the 22nd National Meeting of the Reticuloendothelial Society

Ithaca, New York, August 3–8, 1985

This joint conference will be held on the campus of Cornell University. Scientific sessions will consist of symposia, poster sessions, and poster discussion workshops. Symposium topics include oncogenesis and cell growth regulation in normal and neoplastic cells, development and differentiation of T lymphocytes, neuroendocrine-immune interactions, leukocyte biology of natural host defense mechanisms, and cell biology of natural host defense mechanisms. Financial awards will be given for the best student research papers, for the best young investigator's research paper, and for student travel.

For further information, contact: RES/LCC Conference Office, c/o Dr. Sherwood M. Reichard, Medical College of Georgia, Augusta, GA 30912 (telephone: 404-828-2601).

Fourth World Conference on Lung Cancer

Toronto, Canada, August 25–30, 1985

Sponsored by the International Association for the Study on Lung Cancer and following in the tradition of the three previous world conferences on lung cancer in 1978, 1980, and 1982, the Fourth World Conference will emphasize a multidisciplinary approach to lung cancer. It will highlight current knowledge in biology, prevention, diagnosis, treatment, and supportive care. A full schedule of plenary sessions, mini-symposia, and selected abstracts presented as posters or talks will allow exploration of new and innovative ideas.

The conference has been designated as a Continuing Medical Education activity for 24 credit hours in Category 1 of the Physicians' Recognition Award of the American Medical Association.

For further information, contact: Fourth World Conference on Lung Cancer, Secretariat Office, 342 MacLaren St, Ottawa, Ontario, Canada K2P OM6.

International Meeting on Gynecologic Oncology: Lymphatic Metastases in Gynecological Malignant Tumors

Rome, Italy, September 9–12, 1985

This meeting, which will focus on clinical significance, surgical techniques, and treatment of lymphatic metastases in gynecological malignant tumors, is scheduled to take place immediately before the XIth World Congress of Gynecology and Obstetrics in Berlin, Germany (September 15–20).

In addition to the talks by invited speakers, a limited number of brief communications (in the form of posters), which are strictly related to the topics of the symposium, will be accepted for presentation.

The official languages of the meeting are Italian and English; a simultaneous translation service will be available.

For further information, contact: Prof. Salvatore Mancuso, Department of Obstetrics and Gynaecology, University of Cagliari, Via Ospedale 46, 09100 Cagliari, Italy.

Two Short Courses: Epidemiology and Toxicology

Houston, Texas, October 7–11, 1985

The New England Epidemiology Institute will present two consecutive short courses at the Shamrock Hilton Hotel in Houston. "Principles of Epidemiology" (Oct 7–9) will be taught by Drs. Kenneth Rothman and Stephen Lanes and Mr. Charles Poole. They will present modern concepts in epidemiology and their applications to the study of etiology, natural history of disease, and strategies in preventive medicine and public health. "Regulatory Toxicology" (Oct 10–11) will be taught by Dr. Edward Calabrese. He will discuss how information has been used and should be used in the process of setting standards for drinking water, ambient air, and the workplace.

No previous study of epidemiology, biostatistics, or toxicology is required for either course. Registrants may receive Continuing Medical Education Credits (AMA Category 1) and/or Certification Maintenance Credits from the American Board of Industrial Hygiene.

For further information, contact: New England Epidemiology Institute, Department SC-4, P.O. Box 57, Chestnut Hill, MA 02167 (telephone: 617-734-9100).

Conference on Chemical Modifiers of Cancer Treatment

Clearwater, Florida, October 20–24, 1985

This conference, the fifth in a series of international conferences on radiation sensitization, radioprotectors, and chemical modifiers of cancer treatment, will be held at the Sheraton-Sand Key in Clearwater. The subject of the conference is the use of chemical agents (primarily radiosensitizers, radioprotectors, repair inhibitors, and agents which improve tumor oxygenation) to modify the response of tumor and/or normal tissues to the cytotoxic effects of radiation or chemotherapeutic drugs. Basic scientists in chemistry and biology and clinicians in radiotherapy and chemotherapy will exchange information and ideas on current research, future efforts, and design and implementation of new clinical trials.

The program will consist of eight sessions. It is hoped that financial assistance can be offered to a number of young investigators. The deadline for submission of abstracts, poster presentations, and requests for travel assistance is *June 15, 1985.*

For further information, contact: Ms. Suzanne Bohn, American College of Radiology, 925 Chestnut St, Philadelphia, PA 19107 (telephone: 215-574-3150).

Second International Symposium on Detection and Treatment of Minimal Residual Disease in Acute Leukemia

Rotterdam, The Netherlands, November 6–8, 1985

This symposium is focused on the detection and treatment of subclinical disease in acute leukemia, ie, the small number of neoplastic cells which frequently persist following successful remission induction therapy and cause relapse. The organizers are Drs. Anton Hagenbeek and Bob Löwenberg; topics will include new methods to detect residual leukemia, the value of new chemotherapy programs, and recent accomplishments in clinical bone marrow transplantation.

Abstracts for free poster sessions must be received before *August 1, 1985.*

For further information and abstract forms, contact: J. W. van der Velden, MD, c/o Organizing Committee Leukemia Symposium, The Dr. Daniel den Hoed Cancer Center, P.O. Box 5201, 3008 AE Rotterdam, The Netherlands.

First International Symposium: Multimodality Treatment of Head and Neck Cancer

Genoa, Italy, December 17–18, 1985

The Istituto Nazionale per la Ricerca sul Cancro is organizing this conference as a forum for the most active and prominent investigators in cell kinetics, prognostic factors, diagnosis, staging, evaluation of

response, and single-modality treatment of advanced stages of head and neck cancer. Particular emphasis will be given to combined modalities and second-line treatments.

For further information, contact: M. Merlano, MD, Istituto Nazionale per la Ricerca sul Cancro, Viale Benedetto XV, 16132 Genoa, Italy (telephone: 010-300770).

CORRECTION

In the article "Cisplatin, Doxorubicin, Cyclophosphamide, Lomustine, and Vincristine (PACCO) in the Treatment of Non-Small Cell Bronchogenic Carcinoma" by Robert P. Whitehead, Paul R. Rosenbaum, and P. P. Carbone (Cancer Treat Rep 68:771-773, May 1984), the intervals for median survival time and time to disease progression in the footnote (*) to table 1 are incorrectly described as "ranges" when they are, in fact, 95% confidence intervals for the median.

*U.S. GOVERNMENT PRINTING OFFICE: 1985-461-311:10009

Cancer Treatment Symposia: Proceedings of the Third Annual Sanrocco International Cancer Symposium: Clinical and Biological Evaluation of the Immunomodifiers

The proceedings of the Third Annual Sanrocco International Cancer Symposium, which was held in Sanrocco, Italy, on October 1–4, 1983, are now available. This meeting focused on the potential of biological response modifiers in cancer therapy. Leading tumor immunologists from many countries shared the results of experimental and clinical studies and focused on establishing correlations and cause-effect relationships between the various elements of the biological response and the clinical effects on tumors. Such subjects as monokines, cytokines, and monoclonal antibodies were discussed in depth.

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